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TRENDS IN HEALTH LEGISLATION: LOCAL, STATE, AND NATIONAL*

MILTON I. ROEMER

"Legislation" has a federal connotation these days, and for a very good reason: while there are notable exceptions, the general trend in health legislation has been from a local to a state to a national level. With this trend, there has been a continuous expansion of governmental participation in health service programs. It may be profitable to discuss some of the social changes that have led up to this situation.†

Local health legislation. Early American statutes and ordinances in the health field, as in education and public welfare, were enacted primarily by the cities and towns. Based mainly on the common law police power of government, they were chiefly regulatory and restrictive. Typically, these measures applied to the control of environmental sanitation. In Boston and Salem, Massachusetts, there were enacted in 1678 regulations to control smallpox.[2] In a West Virginia county, recently studied, there were ordinances passed by the City Council between 1838 and 1860 on many sanitary problems: the disposal of rubbish, the slaughtering of animals, the elimination of stagnant water, and the maintenance of outdoor privies.[1] New Haven and other towns in Connecticut have had ordinances in this field on their books since before the American Revolution.[3]

In many Connecticut towns today, the principal legal framework for health activity in the local community relates to the control of sanitary nuisances and similar matters. There may be local statutes regarding the establishment of a Board of Health or the appointment of a Health Officer (usually part-time), but the functions assigned to these authorities are heavily concentrated in the field of sanitation and perhaps the isolation and quarantine of acute communicable diseases. Little will be found locally in the way of legal foundations for maternal and child health services, school health services, industrial health work, mental hygiene, health education, chronic disease control, or the other fields which occupy most of the time of the modern public health agency. Authority for these newer programs has emanated from higher governmental levels.

Even the limited health legislation in local units of government is operative only because of authorities delegated by the state government.

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* From the Department of Public Health, Yale University School of Medicine. Presented at the Annual Meeting of the Connecticut Public Health Association, New Haven, May 17, 1950.

† In this brief discussion, no attempt will be made to review specific health bills under debate in the United States Congress.

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In our country, the state is sovereign and, just as action at the federal level is dependent on reservations of specific powers in the federal Constitution (all other actions remaining state prerogatives), so also action at the local level is dependent on state statute. Such powers have, indeed, been delegated by the states to the towns or counties within them since Colonial times. Little direct state-wide health legislation, however, was enacted until about the 1870's.

State health legislation. The first state public health authority, a board of health, was established in Massachusetts in 1869. Socially and politically the nation was probably ready for state health activities before this, but the Civil War had raised many more pressing problems for the state governments, and state-wide plans to prevent disease through organized action were put on the shelf till hostilities were over.[8] Although the infectiousness of many diseases had been recognized long before this, the 1870's ushered in the golden era of bacteriology. The nature of communicable disease became understood better than ever before and people everywhere learned that town boundaries presented no barriers to the passage of germs. Accordingly, many state regulations designed to control the spread of infectious disease began to be passed. State-wide sanitary codes were enacted and state-wide policies formulated on vaccination against smallpox and quarantine for many communicable diseases. The legal policy which developed was for state health legislation to govern in all localities, except where local ordinances were more demanding.

Another type of regulatory health legislation enacted by the states was designed to protect the public from incompetent medical practitioners. Medical licensure laws had been in effect in certain cities, and later states, since 1760, but most of them were weak and ineffective, and by about 1850 had been repealed.[8] Then in the latter half of the nineteenth century, the states began to pass professional licensure laws which set up examining boards and required candidates to meet certain standards and pass examinations.

While most state health legislation was essentially regulatory over the behavior of private citizens, toward the end of the nineteenth century governmental action was taken by the states to provide certain medical services. Mental hospitals were established and later, sanatoria for tuberculosis. In Connecticut a special hospital and home for veterans was set up, not long after the Civil War. Special institutions were founded, under state authority, for the deaf and the blind. Statutes providing for general medical care for the indigent were, however, primarily local, as one aspect of local measures for the general relief of the "worthy poor."

Around 1910 a new type of legislation, with important health implications, began to be enacted by states. Following the lead of New York and Wisconsin, all the states eventually enacted workmen's compensation laws which provided, among other things, for medical services for workers

injured while on the job.[8] This was a form of compulsory disability and medical care insurance, financed by employers. Medical services under these laws are rendered by private medical practitioners, paid according to fee schedules which are usually embodied in state regulations.

In recent years, many other types of health legislation have been enacted by the states. In general, these statutes have tended to widen gradually the scope of the state public health agency. Laws have been passed requiring vaccination and diphtheria immunization in school children. There are continual modifications of state sanitary codes, with reference to water supplies, sewage disposal, industrial wastes, milk production and distribution, food production and public eating places, the sanitation of tenement houses, and similar matters. Communicable disease regulations are continually being modified; many states give health officers special quarantine and detention authority with respect to tuberculosis and venereal disease. Pre-marital and pre-natal examinations for the detection of syphilis are now required by law in most states.

Federal health legislation. While the federal Constitution does not mention the word "health," national legislation for health purposes has been based on federal powers to regulate interstate and foreign commerce, to care for special groups of federal beneficiaries, to raise taxes and appropriate funds, and in general to promote "the general welfare." But these authorities have been exercised with restraint because of the inherent sovereignty of the states.

The first major federal action in health was the establishment of the Marine Hospital Service in 1798 to provide medical services to merchant seamen. Since shipping involved international trade, this came clearly within the sphere of federal action. Little more in way of federal health authority was undertaken until 1878 when foreign quarantine work was made a federal responsibility, designed to keep communicable disease from entering the country.[10] It was not until 1879 that federal legal action was taken regarding a purely internal health problem: the possible spread of disease from state to state. In this year a National Board of Health was established by Congress for this purpose, based on federal powers to regulate interstate commerce. So jealous were the states of their sovereignty, however, that after only four years the Board was terminated as an encroachment upon states rights. The problem remained, however, and, perhaps with more skillful strategy, responsibility for prevention of the interstate spread of disease was delegated by an act of 1890 to the Marine Hospital Service. In 1902, the name of this agency was appropriately changed to the Public Health and Marine Hospital Service and a decade later to the United States Public Health Service.

Another important regulatory measure was enacted in 1906, the Federal Pure Food and Drug Act. Based on interstate commerce authorities, this legislation aimed to prevent harmful drugs from crossing state lines and to

protect the public against false or misleading claims on labels. It was not until 1939, however, that the law was modified to control dishonest advertising and to require prior examination of drugs for safety before their distribution. Other federal regulatory measures concerned narcotics. Additional special beneficiaries coming under the wing of federal medical service included Indians, drug addicts, and residents of the District of Columbia who were deaf (Columbia School for the Deaf) or psychotic (St. Elizabeth's Hospital). After the first World War the largest group of beneficiaries of all became entitled to federal medical service (with limitations): the nation's war veterans.

Until the early twentieth century all federal and most state health legislation was either regulatory of private actions in the interest of the public welfare or else devoted to medical service for special beneficiaries. Around 1911 a new principle was launched, the principle of federal grants to the states for public health work. The Rockefeller Sanitation Commission had blazed the trail in 1909 by subsidizing the establishment of public health activities in some southern countries. A small sum, initially only $35,000, was made available by Congress to the U.S. Public Health Service for similar public health sanitation activities. These Rural Sanitation Grants were small in immediate impact but tremendous in later effects.[4]

During the first World War, in 1918, the Venereal Disease Control Act was passed by Congress, authorizing special grants by the Public Health Service to the states for VD control work in extra-cantonment areas. The Sheppard-Towner Act for promotion of the Welfare of Maternity and Infancy was enacted in 1921. This law provided funds to the Children's Bureau for grants to the states for organizing well-baby clinics, prenatal clinics, and related activities. It was bitterly opposed by the organized medical profession and was terminated after a few years.[5]

These three measures represented a significant departure in health legislation. They meant the use of governmental taxing powers to provide funds not merely for supporting a "health police force" to restrict private actions, but for supporting aggressive health programs which would seek out disease and attempt to control it. The Sheppard-Towner Act went farthest of all by attempting not simply to prevent communicable disease, but to promote positive health and sound development in mothers and children.

In the halcyon days of the 1920's, these grant-in-aid health programs expired and, under the administrations of Presidents Harding, Coolidge, and Hoover, little new was done in way of federal health activity. Then came the depression. In the black days that ushered in a new administration in 1933, many legal precedents were forgotten. The Federal Emergency Relief Administration almost overnight sent federal funds to local communities throughout the nation to pay for medical care to millions of needy persons. A health service that had been almost exclusively local in support

and control, not even state-administered, became federal in support and regulation. This was a temporary emergency, however, and in 1935 the Social Security Act was passed, returning to the policy of state health administration. The grant-in-aid principle was re-established in the Act's Titles V and VI. The former title on maternal and child health was obviously an extension of the Sheppard-Towner Act and the latter, on general public health, was an extension of the old Rural Sanitation and Venereal Disease Control Acts. So clear was this precedent that Title VI had two distinct sections, with separate appropriations, one for venereal disease and the other for all the rest of public health activity. The sums involved in these programs, while small in relation to national needs, were defined now in millions instead of thousands.

Since then the grant-in-aid policy of the federal government, to stimulate the initiation and development of public health programs in the states, has become extended each year. In 1944, after years of voluntary agency pioneering in the field, federal grants were made specifically for tuberculosis control. In 1946, mental hygiene was added. In 1947, came cancer control and in 1948 dental hygiene. The legislation that initiated these new programs directed to specific disease problems was, strictly speaking, not necessary; the statutory authority for "general public health" in Title VI of the Social Security Act was broad enough to empower expenditures for all these purposes. New organic acts, nevertheless, served to highlight the issues and justify more clearly the appropriation by Congress of additional funds.

The federal grant-in-aid principle has been applied not only to preventive health activities in the states, but also to medical care programs. In 1943, the vocational rehabilitation law (Bardon-La Follette Act of 1920) was amended to include grants for medical services necessary in the correction of static physical or mental defects. During World War II, the Emergency Maternity and Infant Care program provided federal funds to the states for obstetrical services to the wives of servicemen in the lower pay grades and pediatric care to their infants. In 1946, the National Hospital Survey and Construction Act was passed, providing federal grants to the states for the construction of hospitals and health centers by local public or voluntary non-profit agencies. There had been hospital construction under the Public Works Administration in the 1930's and under the Community Facilities (Lanham) Act during the war, but this was the first act in which health service planning—rather than employment relief or war mobilization—was the main objective. This act established also another important principle to be used again in subsequent measures: federal grants to voluntary agencies.

Finally, still another type of federal legislation has been proposed in the last decade, calling not for regulation of health practices nor grants to the states but rather for group budgeting to finance medical services on the

same basis as old-age pensions or unemployment compensation were financed under the Social Security Act. Around 1915, soon after the passage of the first state workmen's compensation laws, social insurance bills for general medical expenses had been introduced in the state legislatures.[11] None of these passed, however, and the issue remained relatively dormant until both the economic depression of the 1930's and the heightened complexity, successes, and costs of medical technology again pushed the question of medical care legislation into prominence.

Numerous federal bills have been introduced in the field of general medical care financing, and the average citizen doubtless hears more about these proposals than about all other health legislation combined. Perhaps it is because of the heat of the public debate and because these measures would affect him most directly and personally. Since 1945, both major political parties have introduced various bills in the field. They are all based on the principle of insurance, either governmentally required insurance or governmental assistance (and regulation) of private insurance plans.

As of this writing, little federal action is expected in the way of general medical care legislation. Meanwhile, bills proposing federal grants to the states for more specialized health purposes continue to be introduced, such as the bill for expanding local public health units, the bill for expanding school health services (including diagnosis and treatment), and the bill for aiding in the organization of rural medical care co-operatives. Of special interest to professional people is the bill to provide federal subsidy to the medical, dental, nursing, and other professional schools. This bill has been passed by the United States Senate, but it has been caught in a web of controversy concerning the adequacy of the nation's supply of physicians and the issue of governmental "controls" over medical education, so that its fate remains to be seen.

Discussion

Today it is evident that most health legislation is at the federal level. What accounts for this shift in emphasis from local and state legal action? Some possible answers may be suggested:

First, in a general way regulatory action at the local and state levels has achieved its purposes. Within the limits of our democratic form of government, police power to control individual behavior in the interest of protecting public health has been amply exercised. Effective promotion of health depends now on educational and personal health service programs beyond the capacity of regulatory action. In a sense, the opportunities for health improvement in America through restrictive or regulatory social action alone have been exhausted.

Secondly, and highly important, there has been a gradual and marked change in the taxing capacity of different levels of government. Local revenues have been derived chiefly from property, usually real estate. With industrialization, the chief source of wealth has passed to industrial production. There have been increasing taxes by state government on the

sale of commodities, like gasoline or tobacco, or even on the general sale of goods (so-called sales taxes), but there are limitations on the scope of taxes a state can reasonably levy on business wealth per se or on general personal income; excessive corporate or income taxes will simply drive individuals and businesses from one state to another. As a result, major taxing powers today have come to rest with the federal government. A computation made in California in 1945 showed that the taxes derived from the people of that state per capita had the following proportions: local taxes $38, state taxes $65, and federal taxes $403.[6] A somewhat similar pattern would be found in any state. Obviously then, new and expanded programs of health service depend mainly on federal legislation. The taxing potentials of state and local government for health purposes have been virtually exhausted.

While these may be the principal explanations of health legislative trends, other factors also probably play a part. The mobility and interdependence of our entire national population have had an important impact on health measures, as on other types of legislation. The importance of this with respect to communicable disease is obvious to all, but it is true for all diseases and disabilities. In so far as health affects productivity and earning power, the vigor of people in one state affects the commerce of people in another. During two World Wars, the Selective Service System taught us that the states with poorer health records could not give their full share of men to the armed forces; citizens of one state suffered from the inadequacies of health resources in another state. Realization of these facts has underscored the need for health legislation on a nation-wide scale.

Finally the general heightening of social and political issues in national political life influences health and medicine, as it does industry, agriculture, or other fields. All issues on which group action is relevant are tending to get more searching consideration in the national Congress. Social pressures from our entire economic system concentrate on the nation's capitol and, in response to these pressures, national legal actions are taken.

With this increasing trend toward federal health legislation, what has happened in local and state legal affairs? Has the federal government "taken over," so that state and local initiative and responsibility have been reduced?

There is no evidence that the increase of federal action has reduced state responsibilities, nor has the increase of state action reduced local responsibilities. There is considerable evidence, on the contrary, that the initiative and expenditures of both state and local governmental units have increased coincident with the increase of federal activities. State governments have appropriated increasingly larger funds for health purposes, and that not solely because of the "matching" requirements of federal grant-in-aid legislation. More than anything else, federal funds have helped to enrich state public health programs, the manifest values of which have summoned increased financial support from state sources. Local expenditures for

health purposes have also increased. State funds for public health work have been granted to local communities since the 1930's, and much of the federal money allotted to the states has been passed on to the counties and cities. Under this stimulation hundreds of new official health agencies have been established in local communities, and programs of widened scope have been launched. Such programs have won stronger financial and moral support from local governmental authorities.

Federal health legislation has, in a word, provided guidance, stimulation, and funds which have strengthened rather than weakened organized state and local health activities. From the viewpoint of sound health administration this is surely as it should be. While legal foundations are increasingly federal, the day-to-day administration of organized health services remains and must remain primarily local.

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TONIC SYMMETRY IN THE INVERTED HUMAN INFANT

ELLIOT R. REINER

Developmental studies of infant behavior have now assumed, especially to the pediatrician and neurologist, a position both of theoretical interest and clinical significance. The impetus for this has been largely through systematic investigations such as those of Gesell and his collaborators at the Yale Clinic of Child Development. A postural reflex of infancy bearing on a facet of their work is described here. No attempt has been made to explain either its obviously involved neurological mechanisms or the possible pathological meaning to variations in this reflex. The findings described should supplement the many contributions of Gesell,[3, 5, 6] Magnus,[8] Minkowski,[10, 11] and Schaltenbrand[12] relating to the symmetro-tonic reflexes. Reviews by Dewey[1] and Dusser de Barenne[2] have discussed other relevant phases of this subject. The present study describes the effect of an inverted body position on the postural symmetry of infancy. The observations made warrant consideration of their theoretical, if not practical significance to the behavior of infancy.

Gesell and Amatruda have reported extended observations on the changing patterns of laterality from 28 foetal weeks through to 28 post-foetal weeks.[5] Their observations were made upon normal infants in supine situations. They indicated the rising and diminishing dominance of the tonic neck reflex in relation to the symmetro-tonic reflex. The abbreviations T.N.R. were used for the tonic neck reflex and S. T. R. for the symmetro-tonic pattern. They observed the T.N.R. starting at 28 foetal weeks, progressing to prevalence at 36 foetal weeks, and persisting through 8 post-foetal weeks, when its recession began. Concurrently with this recession began the rise of the S.T.R. and the coincident submergence of the asymmetry of the T.N.R. The S.T.R. pattern reached its ascendency in the proximity of 20 weeks and was in turn supplanted in several weeks by more elaborate motor coordinations. In view of this waxing and waining of asymmetry and symmetry, it was of interest to find that normal infants up to 36 weeks of age had a constant predominance of tonic symmetry when held in an inverted position. Such a response pattern indicated a specific postural reflex.

An extensive study of the responses of infants to inverted suspension by the feet was reported by McGraw[9] with a different focus of attention. She was mainly concerned with the dominance of flexion or extension of the trunk musculature during development. She did not describe the presence or absence of symmetry, fisting of the hands, or the persistence of definite

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flexion of the elbows in the stage she called spinal extension. The occurrence of crying in her observations was much more frequent, due possibly to the method she employed of suspending the infant by the feet.

In a recent report[7] on the hypertonic infant, the illustrations used showed the reflex symmetry of inversion reported in this paper. It was seen that the S.T.R. was present in both the normal and hypertonic infant. The normal infant was pictured as hanging fairly limp, and the infant with flexion hypertonia as keeping its legs bent. Of 100 infants observed while inverted in the S.T.R. in this present study, none was considered by their physicians to be hypertonic.

Dewey[1] in a survey of the literature on behavior development described the "symmetrical tonic reflexes" as "the stretching of the upper extremities when the neck is bent back and their bending when the head is bent forward." This represented a distinct difference from the tonic symmetry of the inverted infants in this report. The arms remained flexed and tonic in the inverted position regardless of whether the neck was forcibly extended or flexed. No change resulted from supporting the infant's neck and head with the observer's hand. Active or passive rotation of the head to either side did not usually evoke the T.N.R. after the infant was once inverted. It was often noted that the infants resisted the turning of the head.

Results

The S.T.R. in the suspended or inverted position was elicited by holding the infant either by the body or legs and turning him upside down so that the head was lower than all other parts. The spinal column of the younger infants was always supported. The turning procedure, when done evenly and firmly, infrequently produced annoyance and crying. Upon reaching a stationary upside down position, the infants usually clenched their fingers into a fist, or alternately opened and closed them. When suspended by the ankles, the knees were straight and when supported about the body there was only slight flexion of the knees with little tone. The diffuse flexion responses of the crying infant or the relaxation of the sleeping infant obscured the expression of the S.T.R. response in the inverted position.

Fifty-seven of 60 predominantly breast-fed term neonates in the first week of life showed the S.T.R. upon inversion. The three unresponsive infants were apparently normal females three, four, and five days old respectively. The youngest showed evidence of a difficult delivery with a large sub-galeal hematoma associated with molding of the head. The arms of all three infants fell flaccidly at the sides of their heads. A fourth male infant with generalized spasticity was suspected of having some intracranial injury. He showed a sustained clonus of the arms in the S.T.R. position. Promptly or within several seconds of being inverted the 57 who responded assumed positions of the S.T.R. with arms held flexed to head, neck, or thorax. The head was in the midline and was neither flexed nor extended.

FIG. 1. A sleepy, day-old infant shows the characteristic symmetro-tonic reflex posture. The fists are clenched bilaterally but some relaxation has occurred in the infant's right fist.

FIG. 2. A 31-week-old infant maintains the symmetro-tonic reflex. The crossing of the forearms is not significant. In such a position hand sucking may occur secondarily.

FIG. 3. A 40-week-old infant displays bilateral extension while concerned with his inverted position.

The eyes were open and directed forward in a line parallel to the ground. The infants held this position as though fixed in it, and remained in that posture as long as their head was lower than the rest of their body. The longest period an infant was observed in the inverted position was approximately two minutes. Motion picture analyses were made of the responses of some of these infants. A sleepy, term infant in the S.T.R. of inversion is pictured in Figure 1.

Thirty-one normal infants were observed at varying age levels from 10 days to 32 weeks. All manifested the S.T.R. on inversion. Figure 2 illustrates the characteristic S.T.R. posture of one 31-week-old infant in the suspended position. Two abnormal infants were similarly tested. One was a male infant 10 days old who had an undiagnosed exfoliative dermatitis. He did not show the S.T.R. and his arms hung loosely. The second was a male infant observed at 6, 23, and 26 weeks of age. His usual position on inversion at six weeks was a left T.N.R. with the extended arm directed against gravity. At this time he also lacked interest in feeding and was exceptionally irritable and underactive. He continued to show asymmetry at 23 and 26 weeks with a right T.N.R. At this age he had frequent crying spells without any apparent provocation.

Four infants at age levels between 25 and 40 weeks were studied with motion pictures. The S.T.R. on inversion gradually subsided in dominance. It was replaced at 36 weeks by more integrated motor activity and symmetric extension of the arms. Not infrequently the T.N.R. and S.T.R. would recur briefly. Four other infants between 36 and 40 weeks of age had the same postural responses. The absence of the S.T.R. was still more pronounced at 40 weeks (Fig. 3).

Regressive behavior in the inverted position was noted in the 36- to 40-week period. The infant when tired, hungry, or toxic would revert to the earlier T.N.R. and S.T.R. positions. Habit patterns, such as finger sucking, likewise influenced the reflex behavior in the inverted position. One infant, aged 20 weeks, had a tracheotomy tube in place from birth. The mechanical requirements of the tube were such that the infant's head was held facing to the right. Consequently it developed a right T.N.R. as a postural habit. This was maintained in the inverted position, although S.T.R. would replace it if the head randomly entered the midline from the habitual right-sided position.

The most immature subjects were three normal premature infants, all of whom weighed approximately 4 lbs. and had progressed normally in 20 days from an approximate birth weight of 3 lbs. 12 oz. All were noticeably deficient in tone in comparison to the term infants.[4] The first had faint symmetrical flexion of elbows and fisting, with the arms hanging dependently. Observation of this infant was hampered by its extreme drowsiness. The second showed symmetry, but there was a difference in tonus. The right arm was flexed against the thorax, and the left arm, less tonic, was flexed

at the side of the head. The third showed a typical S.T.R. in the inverted position.

Summary

A study was made of 100 infants ranging in maturity from 36 foetal weeks to 40 post-foetal weeks. Observations were made as the infants were held in an inverted position.

Infants under 36 weeks of age when held in this inverted position predominantly showed a symmetro-tonic reflex (S.T.R.) with flexion of elbows and fisting.

The tonic-symmetry of this response took strong ascendance over the asymmetric tonic neck reflex (T.N.R.) normally dominant in the supine position during the first 8 post-foetal weeks.

The disappearance of the symmetro-tonic reflex (S.T.R.) was gradual and incomplete through 40 weeks of age. At this time the S.T.R. had been replaced generally by more integrated motor patterns, and bilateral extension of the arms. Frequently, the earlier T.N.R. or S.T.R. would appear if the child was tired, toxic, or hungry.

Motion pictures were studied at representative age levels in samples of this infant group. Four of the infants were studied at successive age levels in the period between 25 and 40 post-foetal weeks.

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VITAL STAINING OF DAMAGED LIVER CELLS

III. Reactions of Normal and Injured Hepatic Parenchyma of Mice to Rose Bengal*

W. LANE WILLIAMS

Some dyes are excreted almost exclusively by the liver and the rate of their elimination from the circulating blood is used as a test of certain aspects of hepatic function in man. Rose bengal is one of these dyes,[1, 2, 4, 9] and the test in which it is used is similar to the better known procedure utilizing bromsulphalein. Clearance of rose bengal from the blood is known to be retarded in hepatic diseases of man[9] and in chloroform-induced liver damage in dogs.[3] But morphological aspects of hepatic excretion of rose bengal and other "liver function" dyes have received only slight attention[10, 14, 15] (although the hepatic excretion of fluorescein, a related dyestuff, has been studied by means of ultraviolet microscopy[7]). This report presents an account of the intracellular deposition and clearance of rose bengal in normal and carbon tetrachloride-damaged livers of mice.

Methods

The polyhalogens, particularly chloroform and carbon tetrachloride, produce liver damage in many species including the mouse.[5, 12] The procedure followed in producing hepatic injury needs only to be summarized here.

One hundred adult albino mice weighing 25 to 30 grams were given single intraperitoneal injections of a 1% solution (in normal saline) of rose bengal (Color Index no. 779) in a dose of 0.4 cc. per 25 grams of body weight, and were killed 1 to 16 hours later. Twenty of these mice had received a single subcutaneous injection of 0.04 cc. of carbon tetrachloride (in 4 parts of mineral oil) 48 hours prior to injection of rose bengal; and 20, the same dose 24 hours prior to rose bengal. Twenty other mice had been injected with 0.01 cc. of carbon tetrachloride 8 days prior to rose bengal. Forty mice received no treatment other than with rose bengal.

Since weaning, the mice had been fed an adequate diet of fox chow and water. The animals showed no significant alterations in weight during the experiments.

The general tissue reactions, amount of injury, and pattern and rate of necrosis and repair following treatment with carbon tetrachloride had been determined previously in 100 similarly treated mice killed at intervals of 1 to 220 days subsequent to injection of carbon tetrachloride. Livers were fixed and prepared for histological study by methods described previously in detail.[16] In general, the methods were those for paraffin

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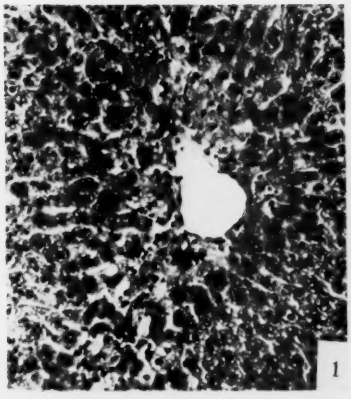
sections except that aqueous and alcoholic reagents were avoided in the procedures subsequent to fixation. Sections cut at 10 micra and mounted as usual on glass slides were studied as follows: (1) without counterstaining; (2) after counterstaining with janus green in acetone;[10] and (3) grossly and microscopically in ultraviolet light, both with and without removal of paraffin, and mounting and covering with glass cover slips. Routine hematoxylin and eosin stained preparations were made. Cytoplasmic basophilia was studied by the veronal acetate buffer-ribonuclease method[11] followed by staining with toluidine blue. Fat was studied in frozen sections by staining with sudan III. Paraffin sections were mounted in 20% alcohol (instead of albumen) on glass slides and were stained in detergent-containing solutions of ponceau 2R at pH 1 to demonstrate cytoplasmic proteins according to the methods of Hyden as used and described by Lagerstedt.[8]

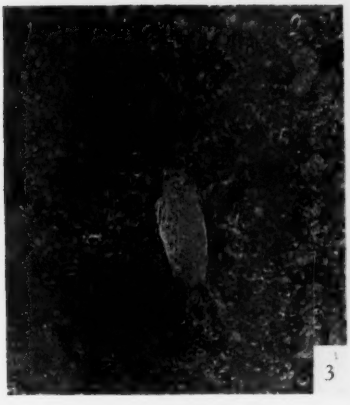
Observations

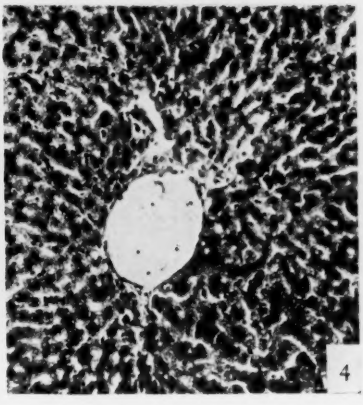
I. Mice receiving 0.04 cc. of carbon tetrachloride 24 to 48 hours prior to injection of rose bengal.

General cytological changes. There was obvious centrilobular damage at 24 hours after injection of the polyhalogen (Fig. 1). In that zone the parenchymal cells had a relatively agranular and hypobasophilic cytoplasm and chromophobic nuclei. Cell membranes appeared intact. Twenty-four hours later cytoplasmic basophilia had decreased further and nuclei were not demonstrable. These damaged cells still existed as structural units with morphologically intact cell membranes which were fairly well demonstrated by the usual cytoplasmic stains and clearly so by janus green. Sections stained with hematoxylin and eosin showed no real evidence of extracentral injury at either 24 or 48 hours after injection of carbon tetrachloride. Methods for demonstrating cytoplasmic basophilia revealed a decrease of this property in the middle zone. Ribonuclease digestion demonstrated that the hypobasophilia was a result of a decrease in cytoplasmic ribopolynucleotides. Frozen sections stained with sudan III showed a considerable deposition of lipid within parenchymal cytoplasm of the middle and peripheral zones. There was obvious necrosis and cytolysis of central cells within 72 hours after injection of the polyhalogen.

Hepatic vital staining and dye clearance. Excretion of rose bengal was obviously retarded in the damaged livers. Grossly, the livers were more deeply stained by rose bengal and remained colored by it for a longer time than the controls. Parenchymal cytoplasm of both normal and injured livers was well stained by the dye within 2 hours after injection. Grossly and microscopically, the greatest concentration of dye was within central cells of damaged livers. The other zones of damaged livers contained less dye but were more heavily stained than any of the zones of the normal livers. Normal livers showed a uniform staining of all zones. The dye was limited to the cytoplasm of parenchymal cells. At all intervals after injection of dye the coloration of normal hepatic cytoplasm was so faint as to be only barely visible in uncounterstained 10 micra sections. In contrast, all of the parenchymal cells of damaged livers showed more obvious staining, and centri-







All figures show central zones and portions of middle zones of hepatic lobules of mouse livers. x150.

Figs. 1, 2, and 3 show condition of liver at 29 hours after one injection of 0.04 cc. of CCl_4, and 5 hours subsequent to injection of rose bengal. Fig. 1 (stained with h. and e.) illustrates typical central damage. Figs. 2 and 3 demonstrate centrilobular deposition of rose bengal. Fig. 2 is unstained except for vital staining of parenchymal cytoplasm by rose bengal. The dark objects are sinusoidal macrophages containing pigment. Fig. 3 is a section counterstained with an acetone solution of janus green which lightly stained cytoplasm and nuclei and combined with the intracytoplasmic rose bengal to produce a purple to lavender color. Nuclei and normal cytoplasm are stained blue-grey.

Fig. 4 (h. and e. stained) shows essentially normal liver 8 days after injection of 0.01 cc. of CCl_4. In such livers the excretion of rose bengal was slightly retarded.

lobular cells were the most heavily stained (Figs. 2 and 3). All zones of the normal livers were cleared of dye within 4 to 6 hours post-injection. In carbon tetrachloride-treated mice rose bengal remained within peripheral and midzonal cells for 5 to 8 hours and in central cells for as long as 14 hours. Table 1 presents the data on vital staining and time required for complete clearance of rose bengal by normal and damaged livers.

TABLE 1

Procedure	*Histological evidence of parenchymal injury*	*Time required (subsequent to i.p. injection) for complete clearance of rose bengal by hepatic parenchymal cells*	
		Central zone of hepatic lobules	*Middle and peripheral zones*
Injection of rose bengal	No injury produced by the dye	4-6 hours	4-6 hours
0.04 cc. of CCl_4 24 hours prior to injection of rose bengal	Centrilobular changes typical of damage by CCl_4	9-11 hours	5-8 hours
0.04 cc. of CCl_4 48 hours prior to injection of rose bengal	Similar to above but more advanced (see text)	11-14 hours	5-6 hours
0.01 cc. of CCl_4 8 days prior to injection of rose bengal	Normal in h. and e. preparations. Slight cytoplasmic hypobasophilia and degranulation of central cells in sections stained with toluidine blue	8-9 hours	4-6 hours

Relation of the dye to hepatic parenchymal cytoplasm. The dye appeared in the cytoplasm of normal and damaged liver cells as a suffusion or diffuse stain and was not segregated into particles or granules. The dye was apparently attached to the granular cytoplasm since it had assumed its shape and distribution. The dye also stained the "intergranular" cytoplasmic areas (the so-called cytoplasmic ground substance) of normal and damaged liver cells, although the staining of larger granules of cytoplasm was more evident, particularly in the photomicrographs (Figs. 2 and 3). Since basophilic granules, most probably ribopolynucleotides, were lacking in the central cells of the carbon tetrachloride-treated mice, it would seem

that the dye was attached to acidophilic granular constituents. An increase in such constituents in the damaged central cells was demonstrated by staining with acid dyes at very low pH[8] (ponceau 2R at pH 1). This may indicate an increase in certain fractions of the cytoplasmic protein complex.[8]

Sections counterstained with an acetone solution of janus green (blue in color) showed that centrilobular cells of the damaged livers contained sufficient rose bengal at 2 to 8 hours after injection to produce a lavender colored cytoplasm as the result of the pink of the vital staining plus the blue of the counterstain (Fig. 3). Normal parenchymal cells never contained sufficient rose bengal to alter the blue color of the janus green counterstain.

Reactions of vitally stained hepatic tissue to ultraviolet light. Dilute aqueous solutions of rose bengal exhibit a green-yellow fluorescence. Solutions of the dye in the formalin-alcohol-acetic acid mixture used for fixation possess a yellow-orange fluorescence. Paraffin sections of normal livers showed no fluorescence at 1 hour after injection of rose bengal, faint yellow at 2 to 3 hours, and no fluorescence after 4 hours. Similar sections of liver from mice receiving 0.04 cc. of carbon tetrachloride 24 hours prior to rose bengal reacted as follows to ultraviolet light: at 1 hour after injection of dye—faint yellow; 2 to 5 hours—orange-yellow; 6 to 8 hours—faint yellow; subsequently—no fluorescence. The fluorescence was limited to central zones during the later stages (after 6 hours).

Rose bengal in extrahepatic sites. Thirty minutes following injection of dye the integument was pink in color. In the controls this discoloration continued for 2 to 3 hours, and in the carbon tetrachloride-treated mice for 3 hours longer. The latter were more obviously tinted. Dye was present in the extrahepatic biliary ducts, gall bladder, duodenum, and pylorus at about the same time it was observed in the liver (obvious gross staining). Within 3 to 4 hours after injection, the intestinal luminal surfaces and contents were heavily stained. Attempts to chemically fix the surface staining of the mucosa of the intestines and gall bladder for histological study were unsuccessful.

Extrahepatic excretion of rose bengal. In both normal and polyhalogen-treated mice there was indication of renal excretion of dye. The kidneys were lightly stained (pink) and the urine was orange in color. In histological preparations the dye was not demonstrated in renal glomeruli or tubules. This may have been due to its small quantity or improper fixation. Since the urine was orange in color, in contrast to the almost cherry red color of the dye within the intestinal tract, it is possible that a contaminant was being excreted by the kidneys. Dilute solutions of rose bengal in buffers at pH ranges of 2 to 8 showed the usual pink to red color. Very concentrated solutions of the dye have a darker red color similar to that observed within the gall bladder and intestine.

II. Mice receiving 0.01 cc. of carbon tetrachloride 8 days prior to injection of rose bengal.

Preliminary studies had shown that one-fourth of this dose of carbon tetrachloride produced cytoplasmic degranulation and hypobasophilia of centrilobular cells within 24 hours. Therefore, the dose used here is well within the toxic range. Eight days following injection of this amount (0.01 cc.) of carbon tetrachloride the livers appeared essentially normal in routine hematoxylin and eosin-stained preparations (Fig. 4), but these livers cleared rose bengal 2 to 3 hours slower than controls and the retention appeared to be limited to central zones (Table 1). Application of the ribonuclease-toluidine blue method indicated that a reduction in basophilic granulation due to a decrease in cytoplasmic ribopolynucleotides still existed within these central areas.

Discussion

Vital staining of damaged liver cells by acid diazo dyes is an "all or none" reaction since such dyes are not deposited in normal hepatic parenchymal cells of mice.[2, 13, 14, 19] The reactions to the phthalein dye, rose bengal, are quite different, as it is excreted by both the normal and injured liver. The eventual, although retarded, clearance of rose bengal by centrilobular cells obviously damaged by carbon tetrachloride, indicates that some degree of excretory activity was still present within these prenecrotic cells. Similarly damaged cells are unable to eliminate diazo dyes, and such dyes remain attached to cytoplasmic remnants until lysis has ensued.[12, 13, 14]

Livers of mice receiving a smaller dose of carbon tetrachloride and injected 8 days later with rose bengal had a relatively normal parenchyma but excreted rose bengal at a retarded rate. This seems to indicate an insufficiency in the excretory ability of these livers. Cytoplasmic hypobasophilia, due to a reduction in ribopolynucleotides, was a morphological concomitant of this excretory insufficiency.

The general histological picture of centrilobular hepatic damage produced by carbon tetrachloride is well known and needs no comment here. More precise cytoplasmic alterations include a decrease in cytoplasmic ribopolynucleotides and a positive alkaline phosphatase reaction—changes which are not specific to damage produced by carbon tetrachloride, but are also characteristic of nutritional deficiencies in rodents and dogs.[8, 17, 18, 20]

It has been usually assumed that extracentral zones are not damaged by carbon tetrachloride. In the present study these zones showed retardation in excretion of rose bengal when it was injected 24 hours after treatment with carbon tetrachloride, but not at 48 hours. At the later period the central zones showed more evidence of damage than 24 hours earlier. This may indicate that at 48 hours the extracentral cells had recovered from, or accommodated to, a possible low-grade or reversible injury that inhibited the excretory mechanism 24 hours earlier. Careful studies of very early

reactions to polyhalogens are needed before these findings can be explained. Cytoplasmic hypobasophilia in the middle zone, and cytoplasmic liposis in middle and peripheral zones were the only morphological indications of abnormality in extracentral areas.

Other reports indicate that in man, and in the dog and rat, rose bengal is excreted exclusively by the liver.[3, 4] In all of the mice, both with and without liver damage, a small amount of dye was excreted by the kidney. This may have been caused either by the relatively large amount of dye injected or by contaminants. It was obvious that the major excretory route was through the hepato-biliary system.

Rose bengal is excellent for study of many aspects of hepatic excretory activity, but the light pink color of the cytoplasmic vital staining produced by it has some disadvantages in cytological studies. This may be offset in part by use of fairly simple methods of ultraviolet microscopy. The presence of greater intracytoplasmic concentrations of rose bengal transiently labels damaged parenchyma and offers some information concerning the rate of performance of one of the exceedingly numerous activities of liver cells.

Summary

In mice, carbon tetrachloride produced hepatic parenchymal damage in which the excretion of rose bengal was significantly retarded. This delay in clearance consisted of retention of dye within the cytoplasm of centrilobular cells and of staining of cytoplasmic constituents by it. Following treatment with the same amount (0.04 cc.) of carbon tetrachloride, the period of dye retention and cytoplasmic staining was longer at 48 hours subsequent to injection of the hydrocarbon than at 24 hours. Evidence of cytoplasmic injury was greater after the longer interval. At 24 hours subsequent to injection of carbon tetrachloride the extracentral zones showed a slightly prolonged interval of dye retention which was not observed at 48 hours. Obvious necrosis and lysis of damaged parenchymal cells did not occur until 72 hours after treatment with carbon tetrachloride. The inhibition in clearance of rose bengal was characteristic of the prenecrotic or precytolytic phase of injury and the eventual excretion of dye by damaged cells indicated the presence of a residual, but defective, excretory mechanism.

A delay in clearance of rose bengal occurred in livers of mice that had received a smaller (0.01 cc.) dose of carbon tetrachloride 8 days prior to the dye. At this time livers exhibited no clear histological evidence of residual injury other than a slight decrease in cytoplasmic granulation and basophilia of some of the centrilobular cells.

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STUDIES ON THE *IN VITRO* DRUG RESISTANCE OF E. COLI TOWARDS MIXTURES OF SULFONAMIDES*

E. GRUNBERG AND D. ELDRIDGE

Although it is a well-known fact that drug resistance towards sulfonamides is easily obtained after a comparatively small number of exposures of the sensitive microorganisms to these drugs *in vitro* or *in vivo*,[1] there is, to our knowledge, no report in the literature concerning the influence that mixtures of different sulfonamides might have on the development of resistant strains.

The wide use of combinations of three sulfonamides, which is due to the better solubility and the curtailing of by-effects particularly in reference to the kidneys,[5,6] made it seem desirable to investigate the influence of sulfonamide combinations on the development of drug resistance of bacteria. The individual components of these mixtures, as far as they were available, were of course studied at the same time. In addition, a new sulfonamide, 3,4-dimethyl-5-sulfanilamidoisoxazole (Gantrisin) was also tested because no experimental data on drug resistance were available for this compound. The development of drug resistance to this compound has apparently not been observed clinically and in one report the absence of this tendency has been specifically stressed.[2] The results of these studies are given in the present paper.

Materials and methods

Two groups of sulfonamide combinations were investigated. The first group comprised five sulfonamide combinations designated A, B, C, D, and E which contained mixtures of sulfonamides of high activity. The first four of this series contained the well-known combination of sulfathiazole, sulfadiazine, and sulfamerazine, while mixture E contained sulfadiazine, sulfamerazine, and sulfamethazine. These sulfonamides, constituting a mixture of 33% of each ingredient, were commercially available products.

Only one representative of a second group was included. This was sulfonamide mixture F which contained besides sulfadiazine and sulfamerazine the soluble N^1-sulfanilylacetamide (sulfacetamide). In addition, the following single sulfonamides were also tested: sulfathiazole, sulfadiazine, sulfamerazine, sulfacetamide, and Gantrisin.*

A strain of E. coli J was used as the test organism[9] in all experiments.

The solutions of the sulfonamide mixtures and the single sulfonamides were made

* From the Chemotherapy Laboratories, Hoffman-La Roche, Inc., Nutley, New Jersey.

* Sulfamethazine was not included in the experimental work since it was unavailable at the time of these studies.

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by dissolving the tablets or powder in alkali, filtering and adjusting to pH 7.0 to 7.5.

The technique described below was used in rendering E. coli resistant to the sulfonamides *in vitro*. The organism was grown in test tubes containing synthetic medium[1] with decreasing concentrations of the sulfonamides or sulfonamide mixtures made up by the serial dilution method. The range of concentrations was from 0.00049 mg./ml. to 1.0 mg./ml. except in the case of sulfacetamide where the highest concentration used was 5.0 mg./ml. After twenty-four hours' incubation, the first tube showing good growth was used for the inoculation of a new experiment and so on until resistance to 1.0 mg./ml. or 5.0 mg./ml. (sulfacetamide) was achieved.

The inoculum consisted of 0.05 ml. of a 10^{-5} dilution of the culture of E. coli (strain J).

The experiments were extended to a period of approximately four weeks or to the time at which full growth was obtained in the highest concentration.

TABLE 1

DRUG RESISTANCE OF E. COLI TOWARDS DIFFERENT SULFONAMIDES IN VITRO

Passage number	*Growth inhibiting concentration in mg./ml.*				
	Sulfathiazole	*Sulfadiazine*	*Sulfamerazine*	*Gantrisin*	*Sulfacetamide*
0	0.00098	0.00195	0.00195	0.00049	0.0195
3	0.0039	0.0039	0.0156	0.0039	0.625
6	0.0312	0.0312	0.0625	0.0312	2.5†
12	0.5	0.125	+1.0	0.0625	....
18	0.5	0.25	....	0.25	....
21	0.5	0.25	....	0.5	....
26	+1.0*	+1.0	....	+1.0	....

* + 1.0 = growth in 1.0 mg./ml.
† Strain was + 5.0 in the seventh passage.

Experimental

In preliminary experiments it was determined how many passages through drug-containing medium were required to render a strain resistant to 1.0 mg./ml. of the single sulfonamides. The results of these experiments are given in Table 1.

The data in Table 1 which represent selected steps in the development of drug resistance show that sulfadiazine, sulfathiazole, and Gantrisin rendered the strain of E. coli J resistant to 1.0 mg./ml. after twenty-six passages. The progression of the loss of sensitivity was in all instances almost identical. Comparatively fast development of drug resistance was, however, observed in two instances. The initially highly active sulfamerazine rendered the strain resistant towards the 1.0 mg./ml. concentrations in only twelve passages. Sulfacetamide appeared not only considerably less active than the other four sulfonamides, but resistance developed so fast that the strain was no longer influenced by a concentration of 0.625 mg./ml. after three passages and was completely resistant to 5.0 mg./ml. after seven passages.

The observations in this table were accurately reproducible. Identical experiments to those given in Table 1 were, for instance, carried out at different times with Gantrisin and sulfacetamide. In an experiment with Gantrisin and E. coli J carried out in May-June, 1946, the initial active concentration was 0.00049 mg./ml. After twelve passages the active concentration was 0.025 mg./ml., after eighteen passages 0.25 mg./ml., and after twenty-six passages the resistant organism grew in a concentration of 1.0 mg./ml. The identical steps in the development of drug resistance in an experiment in January-February, 1950 (not identical with the experiment given in Table 1 which is based on an experiment performed during November-December, 1949) were 0.00098 mg./ml., 0.0625 mg./ml., 0.25 mg./ml., and the culture finally grew in 1.0 mg./ml. after twenty-six passages.

TABLE 2

DRUG RESISTANCE OF E. COLI TOWARDS MIXTURES OF HIGHLY ACTIVE SULFONAMIDES IN VITRO

Passage number	*Growth inhibiting concentration (mg./ml.) of mixtures*				
	A	*B*	*C*	*D*	*E*
0	0.00195	0.00195	0.00195	0.00195	0.0039
3	0.0078	0.0156	0.0039	0.0625	0.0078
6	0.0625	0.125	0.0312	0.125	0.125
12	0.125	0.25	0.0625	+1.0	0.25
18	1.0	0.25	0.125	....	0.5
21	+1.0*	+1.0	0.5	....	0.25
26	....	....	+1.0	....	+1.0

* + 1.0 = growth in 1.0 mg./ml.

The experiment with sulfacetamide as given in Table 1 had been carried out two months previously with identical results. After four passages the strain grew in a concentration of 1.0 mg./ml. At that time this was the highest concentration tested.

A surprisingly fast development of drug resistance towards sulfamerazine was also observed in a second series of experiments with very similar results. The minimal active concentration at the different steps such as passages number 0, 3, 6, 12, and 18 were 0.00195 mg./ml., 0.0312 mg./ml., 0.125 mg./ml., 0.5 mg./ml., and more than 1.0 mg./ml. respectively, which constitutes an almost identical replica of the experiments in Table 1.

The results of drug resistance experiments with the sulfonamide mixtures were, as a rule, very similar to those with the single drugs. Table 2 shows, in condensed form, the development of drug resistance towards the five mixtures which were characterized as mixtures of highly active sulfonamides. The figures given in Table 2 indicate that resistance to the highest

concentration tested in these experiments, namely, 1.0 mg./ml., was obtained in twenty-one to twenty-six passages in increasing concentrations of the drug mixtures. Only one of these mixtures produced a resistant strain much faster despite the fact that this agent, mixture D, contained the same constituents (sulfadiazine, sulfathiazole, and sulfamerazine) as mixtures A, B, and C. The experiment was, therefore, repeated with a freshly prepared solution of mixture D once again starting the exposure of the parent strain of E. coli to the drug. The results of this experiment were as follows:

Passage 0: 0.00098 mg./ml.
Passage 3: 0.0039 mg./ml.
Passage 4: 0.0312 mg./ml.
Passage 6: 0.125 mg./ml.
Passage 9: 0.5 mg./ml.
Passage 10: +1.0 mg./ml.

TABLE 3

DRUG RESISTANCE OF E. COLI TOWARDS MIXTURE F IN VITRO

Passage number	*Growth inhibiting concentration in mg./ml.*	
	Experiment 1	*Experiment 2*
0	0.00049	0.00049
1	0.0039	0.0039
2	0.0039	0.0078
3	0.0078	0.0078
4	0.0156	0.0156
5	0.0156	0.0625
6	0.0312	0.125
7	0.0312	0.125
8	0.0625	0.25
9	0.0625	0.25
10	0.125	0.5
11	0.125	0.5
12	+1.0*	+1.0

* + 1.0 = growth in 1.0 mg./ml.

It seemed, therefore, that the tendency to produce drug resistance more rapidly than other compounds was overcome by the presence of sulfathiazole and sulfadiazine in case of mixtures A, B, and C, but became occasionally manifest, e.g. in mixture D.

The experiments with mixture F appeared particularly interesting because this combination contained two sulfonamides of high initial activity, sulfadiazine and sulfamerazine, and one, sulfacetamide, with low initial activity. From the point of view of development of drug resistance the mixture contained two components with a tendency to rapid appearance of

drug fastness. It seemed doubtful that the presence of sulfadiazine might counteract the influence of the two other components.

Table 3 shows that this was indeed the case. Two experiments carried out with the same strain but two months apart clearly demonstrate that resistance to the final dose of 1.0 mg./ml. was accomplished after twelve passages. The speed with which the strain became drug resistant closely resembled that of sulfamerazine alone, indicating that the presence of sulfadiazine was not sufficient to counteract the influence of two sulfonamides which tend to render a strain resistant rapidly.

Comparative experiments with Gantrisin were included with these tests as well as experiments to show that the sensitivity of the parent strain did not change during the time of these experiments. In fact, the strain of E. coli J has kept its sensitivity to sulfonamides constant for the last four years.

Discussion

Recent experience with combination therapy has shown that combinations of drugs with different sites of attack might produce an interference effect as far as the development of drug resistance is concerned.[4, 10] This possibility appeared remote in the case of sulfonamides, particularly when one considers the fact that resistance towards one sulfonamide tends to overlap to the entire group of these agents. The present study on drug resistance of E. coli induced by exposure to sulfonamides or their mixtures seems to confirm this fact, since mixtures showed the same tendency to render E. coli resistant as did their individual components.

Sulfonamides of high activity and one sulfonamide of comparatively low bacteriostatic effect were included in the experiments on the development of drug resistance of E. coli. The drugs also differed as to the speed with which drug resistance was obtained. If we may adopt the term coined by Paul Ehrlich[8] and later extended by Schnitzer[8] with regard to drug resistance with arsenicals, we could distinguish between compounds of high avidity, e.g. sulfathiazole and sulfadiazine, which produce drug resistance comparatively slowly, and those of low avidity which like sulfacetamide and sulfamerazine induce drug resistance much more rapidly. Drug resistance of a strain of E. coli occurred slowly when the culture was exposed to the first group, namely, sulfonamides of high activity and high avidity. The presence of these drugs in combined preparations was also sufficient to overcome the influence of sulfamerazine which is characterized by high activity and low avidity. One example was found, however, in which a mixture of sulfathiazole, sulfadiazine, and sulfamerazine showed the low degree of avidity characteristic of the last named compound. In one mixture (F) which was a combination of one sulfonamide of high activity and high avidity + one sulfonamide of high activity and low avidity + one of both low activity and low avidity, drug resistance occurred rapidly.

Summary

1. *In vitro* experiments with E. coli carried out for the study of drug resistance towards sulfonamides showed that one can differentiate three types of sulfonamides according to initial bacteriostatic activity and speed of occurrence of maximal resistance: (a) sulfonamides of high initial activity and high avidity, sulfathiazole and sulfadiazine; (b) sulfonamides of high initial activity and low avidity, sulfamerazine; and (c) sulfonamides of both low initial activity and low avidity, sulfacetamide.

2. In the recently developed triple mixture of sulfonamides the presence of two components of high activity and high avidity overcame, as a rule, the influence of one component of low avidity. The presence of two components of low avidity in a triple mixture consistently resulted in the rapid development of a resistant strain.

3. Included in these studies was work with a new sulfonamide, Gantrisin. It belongs to the group of high initial activity and high avidity.

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NUTRITION OF THE MOUSE

IX. Studies on Pyridoxine and Thiouracil*

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The incidence of granulocytopenia in a series of 5,745 cases of thiouracil administration has been reported to be 2.5%,[3] and clinical studies have suggested that pyridoxine in large doses is of value in restoring the blood picture to normal.[4] The present experiments were devised to determine (a) if granulocytopenia could be induced in pyridoxine-deficient and in adequately fed mice by the use of thiouracil, and (b) if pyridoxine could restore the blood picture in neutropenic animals.

Methods

A total of 93 female mice of the C_{57} and A strains were used. The animals were between two and four months old at the beginning of the study, appeared healthy, and showed normal blood counts. They were kept in individual screen-bottom cages in an air-conditioned room. The diets used in the experiments are shown in Table 1. Food and water were supplied ad libitum, unless otherwise specified. Food consumption was determined. The mice were weighed once each week. When it was found that animals fed the thiouracil-containing diets reduced their food intake, a group of mice was given 1.0 gm. of the adequate diet #150 daily. Another group was given two food jars, one containing thiouracil (diet #149) and one without the drug (diet #148). Both food jars contained an amount of diet sufficient to meet the daily food requirement. The amount of each diet consumed was determined. Since reduced food intake would also reduce the consumption of the drug, another group of mice was fed either the adequate diet #150 or the pyridoxine-deficient ration #148. Thiouracil was then administered by stomach tube in doses ranging from 4 to 60 mg. daily. Since the higher doses frequently resulted in death, the usual doses employed were 4, 6, or 8 mg. per day.

Tail blood was used for complete blood counts, including total leucocyte, differential leucocyte counts, hemoglobin concentration, and total erythrocyte counts at the beginning of the experiment and at weekly intervals thereafter. If an animal showed neutropenia (arbitrarily a neutrophil count of 500) on two successive weekly counts, it was either continued on the same regime as a control or was given daily 0.2 mg. of pyridoxine by stomach tube in addition to the previous intake of diet or drug. The majority of animals was autopsied at the end of the experiment, particular note being made of the gross appearance of the thyroid, thymus, liver, spleen, stomach, kidneys, adrenals and lungs.

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Results

Mice fed the complete diet without thiouracil (#150) gained weight slowly during the experiment. The pyridoxine-deficient animals (diet #148) on the other hand showed a steady weight loss. About the 13th week of the experiment they appeared excitable and occasionally went into convulsions. No "running fits," paralysis, or dermatitis was noted. Food intake during the early period was nearly normal. The animals fed the thiouracil-containing diets with or without pyridoxine (#149 and #151) sharply reduced their food intake and lost weight. Many mice died, apparently of starvation. The group fed daily 1.0 gm. of diet #150 also lost a great deal

TABLE 1

COMPOSITION OF DIETS

Basic composition*		
	Casein—vitamin-free	25%
	Dextrose	66%
	Salt mixture‡	5%
	Oil mixture†	2%
	Ruffex	2%

* To prepare the control diet (complete) #150 the following supplement was added per 100 gm. of ration: thiamine 0.5 mg.: riboflavin 1.0 mg., nicotinic acid 1.0 mg., calcium pantothenate 6.0 mg., inositol 100 mg., p-aminobenzoic acid 100 mg., choline 150 mg., folic acid 50 μg., biotin 20 μg., and pyridoxine 10 mg. Diet #148 had the same composition as #150 except that pyridoxine was omitted. Diet #149 was the same as #148 but with 1% thiouracil added. Diet #151 was identical with #150 but with 1% thiouracil added.

† Two gm. of the oil mixture contained 1.8 gm. of corn oil and 0.2 gm. of White's Cod Liver Oil Concentrate. To these ingredients were added 6 mg. of α-tocopherol and 1 mg. of 2-methyl-1,4-naphthoquinone.

of weight; these animals began to succumb during the second week on the reduced food intake. When the mice were offered a choice of diets #148 and #149 (with or without thiouracil), they consumed far more of the thiouracil-free diet. When the drug was administered orally in small doses (4, 6, or 8 mg. per day), it was well tolerated, food consumption remaining approximately normal. These dosages are of the same order of magnitude as those used by Astwood[8] with rats.

Mice which had been maintained on the pyridoxine-deficient diet (#148) for four months showed at autopsy small spleens and slightly reduced lymphoid tissue. Several animals showed enlarged, fatty livers. Thiouracil-treated mice almost uniformly showed enlarged, hyperemic thyroids. The stomachs of animals which died as a result of the large oral doses of thiouracil were distended, while the small intestines were usually empty.

The blood counts carried out at the beginning of the experiment revealed no significant strain differences (Table 2) nor did the blood picture change in those animals maintained on diet #150. The leucocyte counts were found

to vary between wide limits. Mice maintained on the pyridoxine-free diet (#148) showed steadily declining red cell counts and hemoglobin values. Two mice in the group showed a transient neutropenia during the 5th week of the experiment. Table 2 shows the average counts during the 7th and 9th weeks. A similar decline in red cell counts and hemoglobin concentration was noted in animals on the pyridoxine-free, thiouracil-containing diet #149 (Table 2). A modest decrease in white cell counts was noted in these mice. The effect of severe caloric restriction can be seen in the animals fed 1.0 gm. of diet #150 (Table 2). Red cell counts, hemoglobin levels, and white cell

TABLE 2

SUMMARY OF BLOOD COUNTS

	Controls		*Diet #148*		*Diet #149*	*Severe caloric restriction*
	C_{57}	*A*	*7th week*	*9th week*	*6th week*	*2nd week*
Red cell count—millions/mm.³	9.30	9.08	9.06	8.76	8.58	7.67
Hemoglobin—gm.%	15.2	15.4	15.2	11.1	12.8	12.4
White cell count/mm.³	8660	6747	7038	6925	5548	3928
Neutrophils/mm.³	1445	1485	1459	1489	2232	2705
Lymphocytes/mm.³	7153	5215	5579	5436	3302	749
Differential count—%						
Neutrophils	16.9	22.3	20.7	22.5	34.0	80.3
Ring forms	6.1	10.7	6.7	9.5	14.0	33.2
Lymphocytes	82.6	77.3	79.0	76.7	66.0	19.4
Monocytes	0.5	0.3	0	0.7	0.4	0.3
Eosinophils	0.8	0.1	0.3	0	0	0
Basophils	0	0	0	0	0	0

* Diet #148 was without pyridoxine.
† Diet #149 was without pyridoxine but with thiouracil added.

counts (particularly lymphocytes) were sharply diminished. The total neutrophil count was higher than in any other series in these experiments. Mice fed diet #148 and given small oral doses of thiouracil showed a slight drop in hemoglobin levels and white cell counts. Neutrophil counts dropped to a minimum during the 3d, 4th, and 5th weeks with several animals reaching the neutropenic level. When pyridoxine was administered, the neutrophil count usually rose, but many mice showed spontaneous recovery.

Discussion

The effects of pyridoxine deficiency noted in this study were much milder than those observed in other species. No acrodynia was seen in our animals. Similar observations have been made by Foy and Cerecedo[5] and by Morris.[6]

White cell and differential counts did not change. This is in contrast to observations reported by Weir *et al.*[9] who, however, resorted to the use of desoxypyridoxine to induce pyridoxine deficiency. The reversal of the lymphocyte to granulocyte ratio reported by these workers is similar to that noted in our inanition studies. The slight reduction in red cell counts and hemoglobin levels in our pyridoxine-deficient animals suggests that some slight degree of inanition existed. It would seem then that pyridoxine deficiency per se has little if any effect on the blood picture in the mouse under our experimental conditions.

The changes in the blood picture of mice with severely reduced caloric intake are similar to those reported for other species. Boutwell *et al.*,[3] using mice, have reported that caloric restriction reduces the total white cell and lymphocyte counts. This is in good agreement with our observations.

The severe caloric restriction in mice fed thiouracil-containing diets and the relatively normal food intake in mice fed thiouracil by stomach tube point to the latter as the method of choice in studies with thiouracil or similar materials. Clearly, neutropenia could be induced by thiouracil administration (as employed in these studies), but the condition was so transient that no definite picture of the possible beneficial effects of pyridoxine administration could be obtained.[1]

Summary and conclusions

1. No strain differences in the blood pictures of normal mice of the A and C_{57} strains could be observed.

2. Pyridoxine deficiency in the mouse was characterized by weight loss and increased excitability. Food consumption under the conditions of these experiments was nearly normal. No marked changes in the blood picture directly attributable to pyridoxine deficiency were noted.

3. Starvation in mice reduced the red and white cell counts and the hemoglobin concentrations; it reversed the lymphocyte to granulocyte ratio.

4. Oral administration of thiouracil in certain dosages permitted maintenance of normal food intake. Under these conditions transient neutropenia was observed.

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INFLUENCE OF THYROXINE ON THE METABOLISM OF AMINO ACIDS AND PROTEINS DURING FASTING*

H. D. HOBERMAN AND J. GRAFF

Following thyroidectomy the catabolism of nitrogen is accelerated, as indicated by studies both on fed and fasting rats.[1, 2, 3] In an earlier report[3] evidence was presented which showed that after thyroidectomy the rate of amino acid catabolism is elevated, while at the same time, a fall in the rate of conversion of body protein to amino acids occurs. The nitrogen output of fasting hypothyroid animals was found to be slightly greater than normal, indicating that of these two effects of thyroidectomy on the metabolism of nitrogen, the influence on amino acid catabolism is predominant.

The experiments to be reported in this communication deal with the effects of small doses of thyroxine on the amino acid and protein metabolism of fasting thyro-parathyroidectomized rats. By labelling the amino acid reservoir of these animals with isotopic nitrogen (N^{15}), it has been possible to show that the daily injection of 10 μg. of *dl*-thyroxine for 7 days is sufficient to repress the catabolism of the tissue amino acids of fasting hypothyroid animals, while at the same time, an acceleration of protein breakdown occurs.

Experimental

Sixty-day-old male rats of the Sprague-Dawley strain were submitted to thyro-parathyroidectomy by the usual procedure. Twenty-five days after operation six animals received 10 μg. of *dl*-thyroxine† daily by subcutaneous injection, and six rats, reserved as controls, received daily injections of saline. All animals were fed Purina Fox Chow pellets and were provided with drinking bottles of an aqueous solution containing 1.8% calcium lactate and 0.2% calcium chloride. After the 5th day of injection the rats were fasted for 24 hours, and on the 6th day, one ml. per 100 gm. of body weight of a 1% solution (0.85% sodium chloride) of isotopic glycine (30.6 atom % excess) was administered by intraperitoneal injection. The animals were placed separately in metabolism cages and urine was collected for 10 and 48 hours after the administration of N^{15}. Injections of thyroxine and saline were continued during the two experimental days. At the termination of each period of collection of urine, the funnels and cages were rinsed, and the urine and washings made to suitable volumes. After filtration, aliquots were removed for quantitative and isotope analyses of the urinary total nitrogen.

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Results

In an earlier report[1] a method was described for the measurement of the approximate rates of utilization of the tissue amino acids for the formation of urea and the synthesis of body protein during fasting. It was shown that the relationship between the amounts of N^{15} excreted in the urine and the utilization of amino acid nitrogen may be expressed by means of the following equations:

(1) $$\beta = k_1 + k_2 = \frac{2.3}{t} \log \frac{E_i}{E_i - E}$$ (Equation 19[1])

TABLE 1

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF THYRO-PARATHYROIDECTOMIZED RATS

Weight*	E_{10}	E_{48}	C_{48}	Urinary N†	k_1	k_2	k_3P
gm.	*mg.*	*mg.*	*atom per cent excess*	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
270	0.691	0.965	0.245	3.04	6.60	5.98	5.19
302	0.606	0.855	0.256	2.30	5.77	6.57	4.96
284	0.580	0.808	0.276	2.15	5.96	6.65	4.60
284	0.600	0.916	0.256	2.62	5.70	4.93	4.96
290	0.656	0.910	0.254	2.57	6.55	6.25	5.00
266	0.551	0.796	0.313	1.99	5.69	6.09	4.06
Mean				**2.45**	**6.05**	**6.08**	**4.80**
S.E.‡				0.15	0.17	0.38	0.17

* Dose, $a = \frac{\text{weight (to nearest dekagram) x } 0.61}{100}$

† Calculated from 48-hour nitrogen output.

‡ Standard error $= \sqrt{\frac{1}{n(n-1)} \sum_{i=1}^{n} (x_i - \bar{x})^2}$

(2) $$E_i/a = \frac{k_1}{k_1 + k_2}$$ (Derived from equation 17[1])

where E and E_i are the amounts of N^{15} excreted in t and T_i hours, respectively; k_1 is the fraction of amino acid nitrogen which, per hour, is catabolized and excreted; k_2 is the fraction of amino acid nitrogen which, per hour, is incorporated into body protein; and a is the dose of isotopic nitrogen administered. It was also shown that the rate of protein breakdown during fasting is measured by the extent of dilution of the administered N^{15}, and may be calculated from the formula

(3) $$k_3P = 100a/C_iT_i$$

where k_3 is the fraction of body protein nitrogen (P) which, per hour, is converted to amino acid nitrogen, and C_t is the isotope concentration of the urinary nitrogen after collection for T_t hours. In the experiments reported in this and an earlier publication,[1] t and T_t are 10 and 48 hours, respectively.

In Table 1 are recorded the data obtained when the rate of excretion of N^{15} was measured after the administration of isotopic glycine to untreated thyro-parathyroidectomized rats. The results shown are in good agreement with those reported previously,[1] and are in contrast with data obtained from normal animals. In the latter the values of k_1, k_2, and k_3P which have been reported are 3.25% per hour, 6.31% per hour, and 6.31 mg. per 100 gm.

TABLE 2

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF THYRO-PARATHYROIDECTOMIZED RATS RECEIVING THYROXINE

Weight*	E_{10}	E_{48}	C_{48}	Urinary N*	k_1	k_2	k_3P
gm.	*mg.*	*mg.*	*atom per cent excess*	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
282	0.584	0.938	0.207	3.37	5.34	4.39	6.14
310	0.481	0.766	0.255	2.02	4.00	5.87	4.98
304	0.423	0.754	0.204	2.54	4.58	6.48	6.23
300	0.546	0.838	0.232	2.51	4.83	5.71	5.48
288	0.405	0.672	0.225	2.16	3.50	5.72	5.65
294	0.333	0.610	0.222	1.95	2.72	5.17	5.22
Mean				**2.42**	**4.16**	**5.56**	**5.62**
S.E.*				0.21	0.38	0.29	0.20

* Please refer to footnotes of Table 1.

per hour, respectively, while the nitrogen output was 2.11 mg. per 100 gm. per hour. The data obtained in the experiment employing thyroxine-treated animals is shown in Table 2. It is evident that the treatment brought about a significant decline in the rate of amino acid catabolism ($P < 0.01$), and an equally significant acceleration of protein breakdown ($P < 0.01$). The difference between the values of k_2 for the two sets of animals is not significant. It will be noted that no change in the rate of excretion of nitrogen occurred in the treated animals, indicating that the inhibition of amino acid catabolism was not of sufficient magnitude to overcome the concomitant acceleration of protein breakdown.

Discussion

In an earlier report[1] evidence was presented which favored the view that in the absence of thyroid gland the secretion of pituitary growth hormone

is markedly repressed. It was shown that the administration of growth hormone to thyro-parathyroidectomized rats brought about not only a reduction in the level of amino acid catabolism, but also accelerated the incorporation of amino acids into body protein. Since the administration of thyroxine to hypothyroid animals also caused a lowering of the rate of catabolism of amino acids, it would appear that the influence of this hormone on the metabolism of amino acids is mediated by the pituitary gland. On the other hand the effect of thyroxine on the mobilization of amino acids from body protein would appear to be more direct. We have recently found that thyrotropic hormone* brings about a significant acceleration of protein breakdown in hypophysectomized rats.

Since growth hormone causes an increase in the rate of utilization of amino acids for the synthesis of protein, it might be anticipated that treatment with thyroxine, by causing an increase in the output of growth hormone, would also accelerate protein synthesis. That this is not the case may be related to the fact that the incorporation of amino acids into protein is exceedingly sensitive to the secretions of the adrenal cortex. This suggests that even small doses of thyroxine may so raise the level of adrenal activity as to block the effects of an augmented output of growth hormone. It is already well known that large doses of thyroid hormone may bring about hypertrophy of the adrenal glands.[4] Since the adrenal cortex is also concerned with the mobilization of amino acids from body protein, it would be of interest to determine to what extent the adrenal steroids mediate the effect of thyroid activity on this process.

The results of the present study are consistent with those of Rupp *et al.*[3] who have reported that small doses of thyroxine (5-10 μg.) diminish the nitrogen output of force-fed rats, and that this effect appears to depend upon the presence of the pituitary gland.

Summary

By measuring the rate of excretion of N^{15} after the administration of N^{15} glycine to fasting thyro-parathyroidectomized rats, it was found that small doses of thyroxine reduce the level of amino acid catabolism and accelerate the breakdown of body protein.

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DISTAL TUBULAR DYSFUNCTION WITH RENAL CALCIFICATION*

ROBERT E. COOKE AND CHARLES R. KLEEMAN

Since the initial description by Albright *et al.*[3] of the syndrome of diffuse renal calcification associated with changes in renal function, other similar cases have been reported.[1, 2, 4, 5, 6, 11, 18, 19] Metabolic balance studies[1, 3] have demonstrated that the altered renal function in this disease usually consists of an inability of the kidney to exchange hydrogen and ammonium ions for sodium so that the loss of sodium from the body in relation to chloride loss is excessive, and metabolic acidosis results. There is also an associated inability of the kidney to conserve water despite systemic dehydration. Both of these functions—the elaboration of an acid urine and ammonia and the facultative reabsorption of water—are considered to be distal tubular functions.[15, 16, 17, 20, 21]

This case is reported because the history possibly suggests congenital origin of this disturbance. The procedures which were carried out in arriving at a definitive diagnosis may be of some interest.

Case history

R.A.† was admitted for the first time to the Pediatric Service of the New Haven Hospital in February, 1950, at the age of six years, with a provisional diagnosis of primary hyperparathyroidism. The chief complaint was that of failure to grow.

Family history. Both parents were of low normal stature. A female sibling had pyloric stenosis in infancy which was relieved by pyloromyotomy.

Past history. The prenatal course of this child was uneventful. His delivery was spontaneous and normal at full term. The birth weight was seven pounds and one-fourth of an ounce. There was frequent vomiting until nine months of age attributed to pylorospasm; weight gain was slow during that time. The weight at one year was fifteen pounds and there was an acceleration of the weight gain at eighteen months. The intake of vitamins C, D, and A was adequate but at no time excessive.

Present illness. The history relevant to the present illness began almost at birth. During the first few months of life it was noted that the child consumed as much as eight ounces of water between each feeding. This polydipsia continued until admission at which time the water intake was about eight to ten glasses per day in addition to one and one-half quarts of milk. Nocturia of two times per night had been present since toilet training had been begun. Because of albuminuria and retarded growth noted in a routine school physical examination, he was admitted to a neighboring hospital. There, a urine concentration test revealed a specific gravity fixed below 1.010. The serum calcium was said to be 15.0 mg. per 100 cc. The alkaline phosphatase was

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† We are indebted to Dr. Stewart Joslin for referring this case to us.

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6.0 Bodansky units. Roentgenographic examination of the abdomen revealed a marked, diffuse speckled calcification of both kidneys. The intravenous pyelogram revealed normal excretory function with no evidence of obstruction in the urinary tract. Urine cultures were negative. Because of these findings the patient was referred to this department.

Physical examination. The patient was a small, fairly well nourished, reddish haired, white boy who was alert and intelligent. The body weight was 15 kg. and the height was 45¾ inches. The size of this child was that of an average child aged four years and eight months.* The vital signs including blood pressure were normal. There were no other abnormal physical findings except for a Grade I precordial systolic murmur which was considered to be functional and *pes planus* right with moderately severe pronation. The skin was somewhat dry. There were no palpable masses nor enlarged organs in the abdomen.

TABLE 1

SERUM ANALYSES

Date	NPN *mg.%*	CO_2 *mEq./l.*	Cl *mEq./l.*	Na *mEq./l.*	K *mEq./l.*	Ca *mg.%*	P *mg.%*	*Alb. gm.*	*Glob. gm.*	*pH*
March										
1	41	11	112	140	4.7	10.6	4.1	4.3	3.7	
8	40	16	106	137	4.5	11.1	4.8	4.3	3.1	7.32
13	45	16	109	139	4.3	10.2	5.8			
14						10.7				
18	35	25	98	143	4.1	10.0	5.0			
21		25				10.9				

TABLE 2

PLAN OF BALANCE STUDY

Procedure	*Period*			
	I	II	III	IV
Duration, days	5	2	3	3
Diet	Control	Control	Control	Control
Parathyroid extract		50 U. every 16 hrs. i.v.		
Sodium citrate			90 mEq./day	
Sodium acetate				90 mEq./day

Roentgenographic findings. Radiographs of the skull and extremities showed no evidence of osteoporosis or osteitis fibrosa. Dental radiographs revealed no changes in the lamina dura. There was delayed appearance of the centers of ossification of both wrists with no appearance of the centers which are usually present at the age of five years. The kidney shadows were normal in size, shape, and position. There

* The plotting of this data on a Wetzel growth chart indicated that he was in channel A_1 with an isodevelopmental line of 10, placing him in an auxodrome in which ninety-eight per cent of the children of his own age are larger than he and in which sixty-seven per cent of children of age four years and eight months are found.

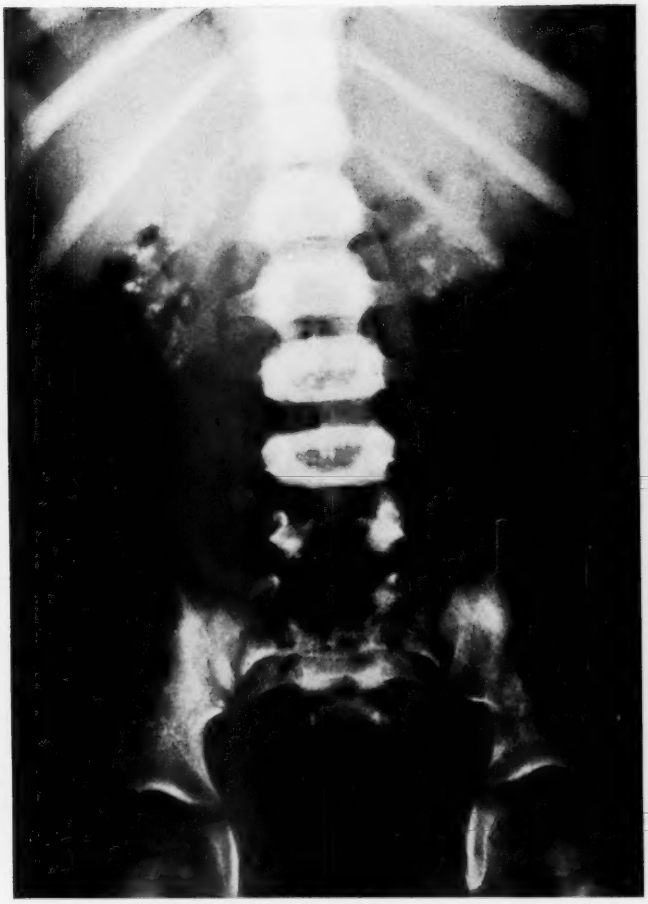

FIG. 1. Radiograph showing marked diffuse bilateral calcification of the renal parenchyma.

FIG. 2. Lateral view of the abdomen showing renal calcification.

was marked focal calcification speckled throughout the kidneys bilaterally with a preponderance in the medullary region. (Figs. 1 and 2.)

Laboratory data. Urinalysis revealed a specific gravity of less than 1.010 despite restriction of fluids for eighteen hours. There was a trace of albumin in the urine. Occasional white blood cells were noted in the urinary sediment. There were no reducing substances in the urine. Qualitative tests for cystine were negative. The Sulkowitz test for calcium in the urine was equivocal. The urine pH was never below 6.8. A phenolsulfonephthalein excretion test revealed ten per cent excretion in fifteen

TABLE 3

BALANCE DATA

Period	*Total weight gm.*	*Water gm.*	*Fat gm.*	N *gm.*	Cl *mM.*	Na *mM.*	K *mM.*	P *mM.*	Ca *mM.*	NH_3 *mM.*	*Tit. acid mM.*	*pH*
I												
5 D.												
Urine	8362			36.3	283	221	341	130	25	85	17	7.0
Stools	336	252	22	4.1	5	1	35	85	166			
Intake	16351	14887	179	63.5	344	258	465	235	204			
Balance	7653		157	23.1	56	36	89	20	13			
II												
2 D.												
Urine	3539			16.9	126	107	135	54	11	34	31	6.8
Stools	127	94	9	1.7	1	1	14	39	70			
Intake	5635	5050	71	26.2	126	94	185	96	83			
Balance	1969		62	7.6	—1	—13	36	3	2			
III												
3 D.												
Urine	4932			21.6	161	251	140	60	9	24	0	7.6
Stools	150	112	11	1.9	1	1	21	45	65			
Intake	8718	7957	90	29.7	173	433	250	114	99			
Balance	3636		79	6.3	10	181	89	9	25			
IV												
3 D.												
Urine	5763			21.9	207	432	200	74	8	7	0	7.6
Stools	245	194	12	2.5	3	3	24	41	78			
Intake	10417	9469	122	38.5	225	452	306	141	124			
Balance	4409		110	14.1	15	17	82	26	38			

minutes and sixty-two per cent in two hours. The hemogram was essentially normal except for a hemoglobin of 12.5 grams per 100 cc. which rose to 14.0 grams during the urine concentration test. The tuberculin test was negative, as were cultures of the blood and urine and serological examination for syphilis.

The analysis of serum on admission revealed a marked metabolic acidosis with hyperchloremia (Table 1). A metabolic balance study was performed to determine the nature of this electrolyte disturbance, using techniques previously described;[8] it consisted of four experimental periods (Table 2).

Results of the balance study

During the five-day control period the presence of hypercalciuria was demonstrated (Table 3). The renal excretion of calcium was 5.0 mM. per day, at least twice the high normal excretion.[18] The retention of chloride exceeded the retention of sodium despite the presence of severe metabolic acidosis (Table 1). The reason for this finding was obvious from the insignificant ammonia and titratable acid production during the period (84.6 and 16.7 mEq., respectively).

In the second period, parathyroid extract was administered intravenously three times without any marked change in the overall electrolyte balance from the control period, despite the immediate change in phosphorus excretion described below.

TABLE 4
PARATHYROID EXTRACT STIMULATION

Time	P *mM./hour*	Ca *mM./hour*	Na *mM./hour*	K *mM./hour*	Cl *mM./hour*
Pre—					
1 h	.836	.147	2.4	2.7	3.1
2 h	.838	.162	1.7	3.6	3.1
3 h	.916	.181	1.8	3.7	3.1
4 h	.965	.160	1.6	3.3	2.4
*Post—					
1 h	1.398	.227	3.3	2.5	3.9
2 h	1.616	.176	2.4	2.5	2.9
3 h	1.577	.197	2.5	2.2	3.0
4 h	1.496	.257	2.2	4.4	3.0
5 h	1.432	.253	2.9	3.3	2.8

*0.5 cc. Parathyroid Extract i.v. (50 units USP).

With the administration of sodium citrate, 90 mEq. per day (Period III), sodium was retained in excess of chloride with a rise in serum carbon dioxide content to 25.0 mEq. per liter. Concomitantly, there was a fall in calciuria to high normal levels. With continuation of alkali therapy (Period IV) the sodium retention fell to that of the chloride retention. Calcium excretion by way of the urine was reduced even further to 2.5 mM. per day. The retention of calcium and phosphorus per day was significantly increased above the control period. The retention of calcium increased from 2.6 mM. per day in the control period to 12.8 mM. on alkali therapy, and the retention of phosphorus increased from 3.9 mM. per day to 8.7 mM.

In an attempt to demonstrate the acute effect of parathyroid extract on renal phosphorus excretion, hourly urines were studied after administration of fifty units of the extract. These showed a significant increase in the renal excretion of phosphorus above the control values (Table 4). Within one

hour after the administration there was a rise in the hourly phosphorus excretion to somewhat less than double the control values. The excretion of phosphorus per hour in the control periods was about 0.89 mM., rose to 1.40 mM. within one hour, and reached a maximum of 1.6 mM. about two hours after the injection of parathyroid extract.

Pitressin hydrochloride (0.5 cc.) was administered intramuscularly at the end of the balance study and the urine volume and specific gravity were measured during a period of controlled water intake. There was no decrease in the hourly rate of urine formation nor increase in urine specific gravity after the injection of antidiuretic substance (Table 5).

TABLE 5

POSTERIOR PITUITARY EXTRACT STIMULATION

Time	*Urine gm.*	*Specific gravity*	*Intake cc.*
Pre—			
1 h	61	1.003	60
2 h	59	1.003	60
3 h	61	1.000	60
*Post—			
1 h	72	1.004	60
2 h	67	1.004	60
3 h	74	1.004	60
4 h	42	1.004	60

*0.5 cc. Pitressin i.m.

Discussion

Before the balance study was performed there seemed to be two diagnostic possibilities. First, the renal calcification might represent a pathological reaction to an old renal injury, such as infection, without progression. Second, this calcification might be progressively increasing because of enhanced renal excretion of calcium. This hypercalciuria could be due to primary hyperparathyroidism or chronic acidosis.

The control period of the balance study definitely established the presence of excessive calcium excretion by way of the urine. This excretion was at least twice the high normal renal excretion of calcium. Furthermore, in this control period it was noted that the production of ammonia and titratable acid was grossly deficient despite the stimulus of a severe systemic metabolic acidosis. This deficient exchange of ammonium and hydrogen ions for sodium prevented the reabsorption of sodium in excess of chloride which would have normally occurred to correct the metabolic acidosis. There was no demonstrable disturbance in potassium metabolism in contrast to the finding of Albright in some of his cases.[1,2] There was adequate

retention of potassium in relation to nitrogen, and hypokaliemia was not observed.

The experiment in which parathyroid extract was administered was carried out in an attempt to rule out primary hyperparathyroidism as the cause of the renal calcification.

The diagnosis of primary hyperparathyroidism is untenable in the presence of repeated normal or high normal serum inorganic phosphorus concentrations unless the renal excretion of phosphorus is impaired. In this case the response to parathyroid extract was similar to that of normal subjects studied by Ellsworth and Howard[10] and by others.[12] In patient R.A. there was a marked increase in the renal excretion of phosphorus within one hour after the administration of parathyroid extract, and this response indicated that the renal pathway for phosphorus excretion was not impaired, and the kidneys could respond to parathyroid hormone stimulation. Consequently, the high normal concentration of inorganic phosphorus in the serum of this patient made the diagnosis of primary hyperparathyroidism unlikely. The renal phosphorus excretion for the whole of Period II was essentially the same as in the control period, despite the intravenous administration of parathyroid extract three times during Period II. This finding is not in conflict with the results of the parathyroid stimulation experiment noted above. The intravenous administration of parathyroid extract produces an immediate increase in phosphorus excretion which may fall to less than control levels of excretion several hours after administration.[7] This negative rebound might explain our findings in Period II of the balance study.

The inability of the end organ—the renal tubule—to respond to antidiuretic substance which was injected further indicates a distal tubular dysfunction as the basis for the obligatory polyuria. The etiology of the tubular dysfunction is not clear. The onset of polydipsia in infancy without other signs or symptoms of acute or chronic renal disease is suggestive of a congenital defect in the enzyme structure of the cells of the distal convoluted tubules of the kidneys with calcification resulting from long maintained hypercalciuria secondary to chronic metabolic acidosis. However, the presence of calcification may not be essential in this syndrome,[9, 11] and secondary renal injury may be avoided if calcification can be prevented. The early diagnosis of this condition would seem to be essential for the prevention of irreversible renal injury by preventing hypercalciuria.

The results of the balance study carried out during the administration of sodium citrate and sodium acetate indicate that by reduction in calciuria renal calcification might be prevented. The mechanism of the reduction in calciuria following alkalinization is not understood at the present time. It has been postulated that the correction of acidosis reduced the concentration of filterable calcium in the serum and thereby reduced the amount of calcium filtered. This explanation is probably unlikely in view of the work

of McLean and Hastings[14] and Dillman and Visscher,[9] since the pH of the serum was not particularly low (pH 7.32) before alkalinization. If a cation exchange system were involved in the distal tubule, the increased sodium reabsorption during alkali therapy might displace calcium inward from the tubular cell and thereby prevent calcification and calciuria. The actual mechanism involved in the reabsorption and excretion of calcium remains to be worked out. Sodium citrate did not seem to be superior to sodium acetate in decreasing the hypercalciuria.

The fairly large positive balances of nitrogen, calcium, and phosphorus suggest that the growth rate of this child may be accelerated by this therapy. These assumptions are confirmed somewhat by the examination of the patient two months after discharge during which period 90 mEq. of sodium acetate were taken daily. At that time were noted a weight gain of eight pounds, which did not seem to be due to sodium retention, as well as an increase in height of one-half inch and a rise of the serum alkaline phosphatase to 18 Bodansky units. The latter probably represents increased osteoblastic activity in the bones.

Summary

A case of distal tubular dysfunction with renal calcification of possible congenital origin is reported. The laboratory procedures which were necessary to confirm the diagnosis are described. The administration of parathyroid extract was used as a means of ruling out primary hyperparathyroidism.

ADDENDUM

Since this paper was submitted for publication, radiographic study of the 4-year-old female sibling was performed. Extensive speckled calcification of both kidneys was noted. Further study of this family will be reported subsequently.

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HEPATIC MANIFESTATIONS OF SARCOIDOSIS AND OTHER GRANULOMATOUS DISEASES*

A Study Based on Histological Examination of Tissue Obtained by Needle Biopsy of the Liver

GERALD KLATSKIN and RAYMOND YESNER

It is evident from both clinical and autopsy studies that sarcoidosis is usually a generalized disease. What was once regarded as a primary disorder of the skin is now known to include a wide variety of apparently unrelated clinical syndromes, having as their common denominator a similar histopathology. The clinical features of the disease, which vary according to the distribution of lesions in the special tissues and organs, are now so well known to clinicians that sarcoidosis is being recognized with increasing frequency. Although studies of autopsy[1-16] and biopsy[17-20] material indicate that the liver is one of the organs most frequently affected, the clinical manifestations of hepatic sarcoidosis have received little attention. This aspect of the disease takes on added significance now that liver tissue is readily available for histological examination by needle biopsy.

Since the etiology of sarcoidosis is still in doubt and since its clinical manifestations are usually not pathognomonic, the diagnosis must of necessity depend on the histological demonstration of typical lesions. When the skin and superficial lymph nodes are not involved, needle biopsy of the liver may be the only practical means of establishing the diagnosis. The chief objections to needle biopsy are that serious, and occasionally fatal, complications may follow the procedure,[21] and that the small samples of tissue obtained may fail to include lesions when they are actually present in the liver; but the dangers are negligible provided the procedure is carried out skillfully and adequate precautions are taken in the selection and postoperative care of patients. In the authors' experience of 650 biopsies there have been relatively few minor, and no fatal, complications. As for errors in sampling, evidence will be presented to show that they are considerably less than might be anticipated from the size of the specimen obtained at biopsy.

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From published data it would appear that sarcoidosis of the liver seldom gives rise to symptoms. A few instances of jaundice[7, 23, 33] and ascites[24] have been reported, but it is not clear whether these were all due to sarcoidosis or to other coincidental causes. Hepatomegaly is a more frequent finding[9, 25, 26] and occasionally poses a difficult diagnostic problem, especially when accompanied by splenomegaly. Banti's syndrome, Hodgkin's disease, and cirrhosis have been frequent diagnostic errors. The problem is somewhat complicated by the fact that the enlargement of the liver in sarcoidosis may be secondary to congestive failure rather than to granulomatous infiltration. The functional status of the liver in sarcoidosis has not been studied systematically, but sporadic reports indicate that it is usually normal or only slightly impaired.[25, 27]

Characteristically, the lesions in the liver occur as scattered, sharply circumscribed, discrete granulomata, predominantly in the portal triad, but occasionally elsewhere in the lobules. Generally, the lobular architecture is little disturbed, and there are few changes in the parenchymal cells. In a few instances, however, an increase in periportal connective tissue has been observed,[16] and in two cases typical cirrhosis has been found at autopsy.[9, 15] Schaumann[15] expressed the opinion that the cirrhosis in his case was related to sarcoidosis, but it is not clear from his description whether the cirrhosis resulted from fibrous tissue replacement of sarcoidal lesions or from the general effects of a chronic debilitating disease. The well-recognized tendency for the lesions of sarcoidosis to heal by fibrous tissue replacement as in the lung suggests that, in some instances at least, cirrhosis may be the end result of healing granulomata.

While the histopathology of sarcoidosis is characteristic, it cannot be regarded as pathognomonic, since sarcoid-like lesions can be produced by a variety of agents, of which the unknown cause of sarcoidosis is only one.[28] It is obvious, therefore, that the diagnosis of sarcoidosis cannot be based on the histological findings in the liver alone, unless a number of other diseases have been excluded. Of these brucellosis and tuberculosis constitute the most frequent differential diagnostic problems. However, granulomata of the liver have also been described in a variety of diseases, including erythema nodosum,[29] beryllium intoxication,[29] syphilis,[30] tularemia,[31] histoplasmosis,[32, 33, 34] blastomycosis,[35, 36] and leprosy.[37] The differentiation of sarcoidosis from these diseases is based on histological criteria in some instances, on the demonstration of specific etiologic agents in others, and on clinical features in the remainder. In some cases the diagnosis cannot be established with certainty even after characteristic granulomata have been demonstrated histologically, and appropriate clinical and bacteriological studies carried out. The complexity of the diagnostic problem stems from the uncertainties regarding the etiology of sarcoidosis. The disease has been variously described as a form of tuberculosis,[10, 38, 39] as a virus infection,[7, 40, 41] and as a characteristic but non-specific response to a variety of etiological agents, including the tubercle bacillus, the brucella organism, beryllium, and possibly others.[42]

The following is a report of an investigation of the clinical, functional, and histological status of the liver in sarcoidosis, and in a number of other diseases known to produce similar granulomata in the liver. Its objectives were: (a) to determine the frequency and clinical significance of hepatic lesions, and to evaluate needle biopsy of the liver as a diagnostic measure in sarcoidosis, and (b) to determine whether the hepatic lesions of sarcoidosis can be differentiated from those of other granulomatous diseases.

Materials and methods

All subjects admitted to the hospital with a diagnosis of proved or suspected sarcoidosis were included in the study. The following procedures were carried out

routinely: (a) a careful physical examination with special emphasis on the state of the liver; (b) blood count, urine analysis, and serological (Mazzini) test for syphilis; (c) a group of liver function tests[45]; (d) serum protein determination; (e) one or more intracutaneous tests with Purified Protein Derivative (PPD first strength = 0.00002 mg., second strength = 0.005 mg.); (f) x-ray examination of the chest and extremities, and (g) needle biopsy of the liver.

TABLE 1

HEPATIC GRANULOMATA DEMONSTRATED IN 650 NEEDLE BIOPSIES

	No. biopsied	*Granulomata present*
Sarcoidosis—total	**20**	**15**
histologically confirmed	15	15
presumptive diagnosis	5	0
Tuberculosis—total	**18**	**7**
acute miliary	4	4
pulmonary	8	2
glandular	3	0
osseous*	2	1
renal	1	0
Erythema nodosum—total	3	2
tuberculin-negative	2	1
tuberculin-positive	1	1
Brucellosis—total	2	**1**
active	1	1
inactive	1	0
Beryllium intoxication—total	**1**	**0**
Viral infections—total	**4**	**3**
infectious mononucleosis	2	1
influenza B	1	1
type unknown	1	1
Mycotic infections—total	**2**	**1**
histoplasmosis	1	0
actinomycosis	1	1

* Case 23 with osseous tuberculosis and tuberculin-positive erythema nodosum included in both groups.

The Vim-Silverman needle[44] and the intercostal approach were employed in obtaining specimens of liver tissue. The tissue was usually fixed in Carnoy's solution, occasionally in 10 per cent formalin, imbedded in paraffin in the usual manner, sectioned at 5 microns, and stained with a modification of Masson's trichrome stain, with hematoxylin-eosin, and by the Ziehl-Neelsen technique for acid-fast bacilli. Occasionally sections were also stained for reticulum by Laidlaw's technique. If no lesions were demonstrable in the first set of slides examined, the block of tissue was sectioned serially in its entirety and re-examined. In several instances, where the lesions found were not typical, a second biopsy was performed.

Similar clinical and histological studies were carried out in all subjects with known or suspected granulomatous disease of other types (Table 1). Included in this group

TABLE 2

CLINICAL FEATURES OF HISTOLOGICALLY-CONFIRMED CASES OF SARCOIDOSIS

Case no.	Sex	Age	Race	Chief complaints	Symptoms: Duration	Symptoms: Wt. loss‡	Symptoms: Max. fever	Distribution of lesions: Skin	Periph. nodes	Thorac. nodes	Lungs	Hepato-megaly	Spleno-megaly	Bone	Eye	Paro-tids	Miscel-laneous	Complications
1	F	34	N	malaise, cough pain in joints	5y.	4	102.4	++*	+*	++	+++	++*	P*	0	0	0	?heart	Banti's synd., splenectomy cholelithiasis
2	F	32	N	fatigue, dyspnea, chest pain	1½m.	3	102.8	0	+	+++	++	++*	0	0	0	0	?kidney	
3	M	54	W	blur. vis., weakness, vomiting, cough, dyspnea	1y.	20	102.8	0	0	0	+	++*	++	0	++	0	?heart	
4	F	43	N	none†	?	0	0	0	0	+++	0	+*	0	0	0	0	0	fibrocalcific tb. rt. apex
5	F	11	N	weakness, abdom. pain, fever	10m.	10	104.	0	++*	0	0	+++*	+++	0	0	0	0	
6	F	32	N	chest pain, fever, cough	2½y.	10	100.5	0	±	+++	0	+*	0	0	0	0	0	syphilis, treated
7	M	33	W	none†	?	0	0	0	0	+++	0	0*	0	0	0	0	0	
8	M	32	N	painful eyes, blurred vision	3wk.	0	0	0	0	++	0	0*	0	0	+++	0	lachry-mals*	glaucoma

9	M	26	W	dyspnea, cough	2y.	6	0	0	+	++	++	0*	0	0	0	0	0	
10	M	41	N	fatigability, anorexia†	?	20	0	0	0	++	0	+*	0	0	0	0	0	fistula-in-ano
11	M	52	W	weakness, finger-cramps, cough, chest pain, dysp.	3y.	25	104.4	0	++	+	0	+*	+	0	0	0	marrow*	pericarditis
12	M	60	W	weakness, dyspnea, cough, urin. frequency	3y.	35	0	0	P*	0	+++	++*	0	0	0	0	0	renal failure metastatic calcification, osteoporosis, compression fractures vertebrae
13	F	34	W	fatigability	6y.	0	99.6	? P	±	0	0	+*	+	0	P	0	0	choroidal deposits
14	M	29	W	fatigability, sweats†	6m.	16	0	0	++	++	0	++*	++	0	0	0	0	thrombosed hemorrhoids rhoids
15	F	34	W	parotid swelling, fever, cough, dysp.	3y.	0	102.	?+	±	+	++	0*	0	0	0	+++	0	

* Sarcoids demonstrated histologically.

† Hospitalized for investigation of abnormal pulmonary findings discovered during routine x-ray examination.

‡ Maximal weight loss, in pounds.

± Palpable lymph nodes regarded as normal; + = slight, ++ = moderate, +++ = marked enlargement.

P Lesions present in the past.

were four subjects with outspoken acute or chronic liver disease, who on routine liver biopsy exhibited typical sarcoidal lesions. The diagnosis of sarcoidosis had not been suspected in any of these, nor could evidence of other similar lesions be found on subsequent investigation.

The criteria used in establishing the diagnosis of sarcoidosis were: (a) the demonstration of typical lesions histologically, (b) a clinical picture compatible with the disease, and (c) the exclusion of specific etiological agents known to produce similar lesions. These were chosen on the assumption that the etiology of the disease is still unknown. Subjects with clinical or histological features of sarcoidosis, but with evidence of active tuberculosis, brucellosis, erythema nodosum, or beryllium intoxication were considered separately, since the relationship of these conditions to sarcoidosis is still a matter of dispute.

Every effort was made to exclude tuberculosis and brucellosis by appropriate cultural and serological studies. When pulmonary lesions were demonstrated by x-ray, sputum or gastric washings were concentrated and examined for tubercle bacilli by staining, by culture on Dubos media, or by guinea-pig inoculation. Only in case 3, with pulmonary lesions suggestive of early pneumonoconiosis, was it not possible to obtain a suitable specimen of sputum or gastric washings for study. One or more blood cultures for brucella organisms were obtained in each of the subjects exhibiting significant fever. Tests for brucella agglutinins were carried out in most of the group. Where lymphadenopathy was a prominent feature, or when the blood picture suggested infectious mononucleosis, tests for heterophile antibodies were performed. In a few instances skin tests for histoplasmosis and coccidioidomycosis were carried out.

Significantly enlarged lymph nodes and suspicious skin lesions were biopsied wherever possible. In a number of instances the bone-marrow, and in one case each the lachrymal gland, the parotid gland, and the spleen were examined microscopically.

Results—Sarcoidosis

The diagnosis of sarcoidosis was suspected clinically in 33 patients admitted to the hospital during the period of investigation. The disease could be excluded with reasonable certainty in 13, i.e., by the demonstration of tubercle bacilli in 5, and by the demonstrations of other causes for the clinical manifestations in 8. Of the remaining 20 cases, histological confirmation of the diagnosis was obtained in 15 (cases 1 to 15), but was lacking in 5.

The clinical and laboratory features of the histologically-confirmed group are presented in Tables 2 and 3. In general they did not differ significantly from those described in other large series.[9, 13, 45]

Clinical evidence of liver disease (Table 4). Hepatomegaly was a frequent finding, occurring in 11 of the 15 confirmed cases. This appeared to be related to the presence of granulomata, since it was not found in any of the 5 cases of probable sarcoidosis without hepatic lesions. However, 4 cases with hepatic granulomata had no hepatomegaly, and there was a poor correlation between the number of lesions demonstrated histologically and the degree of enlargement. In none could the enlargement be attributed to associated congestive failure. As a rule, the liver was only moderately enlarged, soft, and non-tender, and in only one instance, case 5, was it sufficiently enlarged and tender to suggest a primary disorder of the liver.

TABLE 3

LABORATORY DATA ON HISTOLOGICALLY-CONFIRMED CASES OF SARCOIDOSIS

Case no.	Hematology								Tuberculin		Serum chemistry					Serology			Bacteriology			Histology				
	R.b.c.	Hemoglobin	W.b.c.	P.m.n.	Lymph	Mono.	Eos.	Sed. rate	PPD 1	PPD 2	Proteins	Albumin	Globulin	Ca	P	Brucella	Heterophile	Mazzini	Sputum	Gast. Wash.	Blood	Liver	Nodes	Skin	Spleen	Marrow
	x10⁶/ cmm.	gm./ 100 cc.	x10³/ cmm.	per cent				mm. 1 hr.			grams per 100 cc.			mg. per 100 cc.												
1	3.84	12.0	12.9†	74	19	3	0	—	+++	—	8.94	2.41	6.53	10.29	3.63	0	—	+++	0	—	—	+	+	+	+	0
2	4.38	12.5	7.4	80	9	6	5	32	0	0	7.09	2.95	4.14	—	—	0	—	0	0	—	0	+	0	—	—	—
3	3.73	11.0	3.8	72	12	16	0	43	0	+	6.73	2.59	4.14	—	—	0	—	0	—	—	0	+	—	—	—	0
4	4.68	12.0	7.7	57	41	—	2	34	0	0	7.88	3.54	4.34	—	—	—	—	0	0	0	—	+	—	—	—	—
5	4.90	12.0	3.9	67	25	6	2	—	0	0	8.40	3.00	5.40	10.20	5.00	0	0	0	—	—	0	+	+	—	—	0
6	3.52	11.0	5.0	64	33	3	0	—	++	—	8.22	3.37	4.85	—	—	0	—	++‡	0	—	0	+	—	—	—	—
7	4.45	14.0	6.4	71	26	3	0	23	0	+++	7.56	4.14	3.42	—	—	—	0	0	—	—	—	+	—	—	—	—
8	—	12.5	2.8	46	46	2	6	16	0	++	7.80	5.30	2.50	—	—	—	—	0	—	—	—	+*	—	—	—	—
9	—	15.5	4.5	64	23	5	7	14	0	0	7.50	—	—	9.80	4.10	—	—	0	0	—	—	+	0	—	—	—
10	4.11	13.0	5.9	64	31	0	5	—	0	0	8.50	—	—	—	—	—	—	0	—	0	—	+	—	—	—	0
11	—	11.0	10.3	66	25	4	5	—	0	+	7.40	3.20	4.20	9.30	3.40	0	0	0	—	0	0	+	0	—	—	+
12	3.60	13.0	8.9	78	18	4	0	—	0	0	7.01	3.58	3.43	12.89	5.16	0	—	0	—	0	0	+	+	—	—	—
13	4.03	13.0	12.5	59	37	4	0	—	0	+	6.26	4.44	1.82	—	—	0	0	0	—	—	0	+	—	—	—	0
14	4.64	13.5	6.9	65	31	0	4	5	+	—	7.00	—	—	—	—	—	0	0	—	0	—	+	0	—	—	—
15	3.81	11.0	7.0	62	34	4	0	—	0	+++	6.34	3.00	3.34	—	—	0	—	0	0	—	0	+	—	—	—	—

+ Under heading Histology signifies presence of sarcoid lesions demonstrated histologically; elsewhere +, ++ and +++ signify mild, moderate and strong reaction, respectively.

* Biopsy of lachrymal gland also positive for granulomata.

† Leukopenia on previous admission, before splenectomy.

‡ History of treated syphilis.

Tenderness was evident in 2 other cases, and the liver was described as firm in a third.

Splenomegaly occurred in 6 of the 11 subjects with hepatomegaly, and was accompanied by leukopenia and mild anemia in 3 (cases 1, 3, 5), suggesting Banti's syndrome. In one of these (case 1), a splenectomy had been performed 5 years prior to this investigation. In 5 of the 6 cases with splenomegaly and hepatomegaly the diagnosis of Hodgkin's disease or malignant lymphoma had been suspected.

Except for mild jaundice in case 5, and splenomegaly as indicated, there were no other collateral signs of liver disease, such as spider nevi, palmar erythema, increased collateral venous circulation, ascites or edema, in any of the group. X-ray examination of the gastrointestinal tract was carried out in 6 subjects, but no esophageal varices or other abnormalities were demonstrated, except for coincidental cholelithiasis in case 1.

Gastrointestinal symptoms occurred in all but 4 of the cases, but these could not be correlated with the presence of hepatomegaly, nor with the number of hepatic granulomata demonstrated histologically. Moreover, similar symptoms were present in 2 of the 5 unconfirmed cases of sarcoidosis, who had neither hepatomegaly nor demonstrable hepatic granulomata, suggesting that most of the symptoms were due to the general constitutional effects of the disease rather than to specific hepatic lesions. Only one subject, case 5, had mild jaundice which appeared to be directly related to extensive granulomatous involvement of the liver and marked hepatomegaly. Anorexia was present in approximately half the group, and nausea, vomiting, or indigestion in a third each. Five subjects complained of abdominal pain, but in only one (case 5) could it be attributed to enlargement and tenderness of the liver.

Hepatic function. The results of the tests of hepatic function are summarized in Table 4. One or more abnormalities were observed in 12 of the 15 cases. On the whole they indicated only minor impairment of function, and, in several instances, were probably related to alterations in the serum protein pattern rather than to hepatic dysfunction. Only in case 5 did the results suggest moderately severe hepatic damage. In general there was a poor correlation between the results of the tests and the presence of hepatomegaly or the number of granulomata demonstrated histologically. However, no abnormalities of hepatic function were observed in the 3 cases tested with unconfirmed sarcoidosis and without hepatic granulomata.

The serum bilirubin was increased significantly in case 5, and, to a lesser degree, in case 3. Significant bromsulphthalein retention occurred in 5 of the 13 cases tested, but was minor in one, and only moderate in the others. Thymol turbidity was increased slightly in 6, and the cephalin-cholesterol flocculation test was mildly to moderately positive in 5 of the 13 cases tested. These abnormalities appeared to be better correlated with high serum globulin concentrations than with evidence of liver damage, judged

TABLE 4

EVIDENCE OF LIVER DISEASE IN HISTOLOGICALLY-CONFIRMED CASES OF SARCOIDOSIS

Case no.	*Liver*				*Symptoms*							*X-ray*		*Liver function tests*						
	Biopsy	*Enlargement*	*Tenderness*	*Consistency*	*Splenomegaly*	*Anorexia*	*Indigestion*	*Diarrhea, constip.*	*Abd. pain*	*Nausea, vomit.*	*Jaundice*	*G. I.*	*Gall bladder*	*One min. bilirub.*	*Total bilirub.*	*Ceph. flocc.*	*Thym. turb.*	*BSP*	*Prothrombin*	*Alk. phosph.*
1	+	++	0	soft	P	++	+	0	+	++	0	0	stones	—	1.00	2+	*7.5*	*36./*	*60*	8.9K
2	+++	++	0	soft	0	0	+	C+	+	0	0	—	—	0.27	0.75	1+	2.0	—	—	—
3	++	++	++	soft	++	++	0	0	0	++	0	0	0	0.29	*1.22*	1+	3.0	*/18.6*	*55*	4.89
4	+	+	0	soft	0	0	0	0	0	0	0	0	0	0.19	1.04	1+	*6.0*	/6.0	100	4.02
5	+++	+++	++	soft	+++	++	0	0	+++	++	+	—	—	*0.53*	*3.99*	*2+*	*6.0*	*/32.7*	*50*	*56.92*
6	++	+	0	firm	0	0	0	0	0	0	0	—	—	0.19	0.24	*3+*	*7.5*	/3.3	72	4.84
7	+++	0	0	—	0	0	0	0	0	0	0	—	—	0.09	0.60	1+	*8.8*	/5.9	72	*6.50*
8	+	0	0	—	0	0	0	0	0	0	0	—	—	0.20	0.40	0	2.0	/5.5	100	4.80
9	+	0	0	—	0	0	++	C+	+	+	0	hyper-motility	0	—	—	—	—	—	90	2.90
10	+	+	0	soft	0	+	0	0	0	0	0	0	0	0.10	0.20	0	3.8	*/7.0*	*48*	3.90
11	++	+	0	soft	+	0	0	C++	0	0	0	0	0	—	—	2+	1.2	/4.0	87	*10.60*
12	+	++	0	soft	0	+	0	0	0	0	0	—	—	0.09	*0.72*	3+	*7.7*	/5.5	100	*5.30*
13	+	+	0	soft	+	0	+	d+	0	0	0	—	—	0.03	*0.27*	0	0	6.0/	74	*6.40*
14	+	++	+	soft	++	++	0	0	0	0	0	—	—	0.10	0.30	0	1.0	0	*68*	2.50
15	+	0	0	—	0	+	+	0	++	n+	0	—	—	0.17	0.52	0	3.0	*/19.2*	*62*	3.24

Biopsy: + = 1-5 granulomata, ++ = 6-10 granulomata, +++ = > 10 granulomata per section.
Physical findings and symptoms: + = slight, ++ = moderate, +++ = marked.
Liver function tests: abnormal values *italicized*. Normal values = 1-minute serum bilirubin < 0.30 mg. per cent; total serum bilirubin < 1.2 mg. per cent; cephalin-cholesterol flocculation < 2+ in 48 hours; thymol turbidity < 5.0 units; bromsulphalein retention 10%/6%, or less, 30 min./45 min. after injection of 5 mg./kg.; prothrombin > 70 per cent of normal control; alkaline phosphatase < 5.0 units (Bodansky), or < 15.0 K units (King-Armstrong).

by the serum albumin level and bromsulphthalein retention. The prothrombin level was decreased in 6 subjects, but was below 50 per cent of normal in only one. In every instance it was possible to raise the level by the administration of vitamin K.

The serum albumin level was below 3.5 grams per cent in 7, and below 3.0 grams in 3 of the 12 subjects tested. In all but one this was associated with an abnormally high globulin level, exceeding 4.0 grams per cent. These alterations were poorly correlated with the number of hepatic granulomata demonstrated histologically and with other evidence of hepatic dysfunction, suggesting that they were, in part at least, due to extra-hepatic factors.

The serum alkaline phosphatase concentration was increased in 5 subjects, but in 3 the increase was of only borderline significance. Case 5, with the most extensive liver damage, had a level of 56.92 Bodansky units associated with a mild degree of jaundice. However, 5 months earlier, before the appearance of jaundice and when the serum bilirubin concentration was within normal limits, the alkaline phosphatase level was 44.03 units. This degree of hyperphosphatasemia is unusual in liver disease, except in the presence of severe obstructive jaundice or hepatic malignancy, and suggested the possibility of bone lesions. However, x-ray studies and bone-marrow biopsy failed to disclose evidence of sarcoidosis. It is probable, therefore, that the hyperphosphatasemia in this case was due to granulomatous infiltration of the liver. In case 11 the alkaline phosphatase level was 10.60 units. Although x-ray examination of the bones failed to reveal cystic areas, a bone-marrow biopsy demonstrated the presence of granulomata. It is difficult to determine whether the hyperphosphatasemia in this case was due to bone or liver involvement, since many granulomata were also present in the liver. In neither case 5 nor 11 was the serum calcium elevated. On the other hand, in case 12 there was a significant hypercalcemia, associated with decalcification of the bones, metastatic calcification of the soft tissues, and renal failure, but the serum alkaline phosphatase level remained low (5.30 Bodansky units). Harrell and Fisher,[27] who first called attention to the occurrence of hyperphosphatasemia in sarcoidosis, observed the same lack of correlation between the serum phosphatase level and the presence of bone lesions or hypercalcemia, and suggested that the former might, therefore, be related to involvement of the liver. The present findings would appear to corroborate this conclusion.

Liver biopsy and other diagnostic measures. Liver biopsy revealed granulomata in all 15 confirmed cases (Tables 3 and 5). In case 10 the first biopsy disclosed the presence of intrasinusoidal collections of epithelioid cells suggestive but not typical of sarcoidal lesions. However, classical lesions were demonstrable in a second biopsy specimen obtained 2 weeks later. In case 11 typical lesions were demonstrated in the first biopsy specimen, but not in a second obtained a month later. This was the only case in

the series in which the diagnosis of sarcoidosis had not been suspected before liver biopsy.

No hepatic lesions were demonstrated in the five presumptive cases of sarcoidosis. Unfortunately, the biopsy was not repeated in any of these subjects. Only one had enlarged peripheral lymph nodes and suspicious skin lesions. Biopsy of both these tissues failed to reveal granulomata. A bone-marrow biopsy in another case was likewise negative.

Histological confirmation of the diagnosis of sarcoidosis was based on the liver biopsy findings in 12 of the 15 cases. In the remaining 3, the liver biopsies were positive, but the diagnosis had been established by an earlier splenectomy in case 1, and by lymph-node biopsy in cases 5 and 12. The liver biopsy findings were later confirmed in other tissues in 2 of the 12 subjects in whom the diagnosis was based on hepatic granulomata (case 8, lachrymal gland; case 11, bone-marrow), but no confirmatory lesions could be found in 4 lymph-node and 3 bone-marrow biopsies carried out in the remaining 10. Thus, histological confirmation of the diagnosis rested on the liver biopsy findings alone in 10 of the 15 cases, even though all accessible, suspiciously involved tissues were excised for histological study. However, 3 of these subjects (cases 6, 13, 15) had small palpable lymph nodes which were not regarded as abnormal, and which, therefore, were not biopsied. Some of these may have contained granulomata, in view of reports that apparently normal lymph nodes frequently contain diagnostic lesions.[15] Tonsillar biopsy has also been recommended as a useful diagnostic measure,[15] but none was carried out in this series.

If it is assumed that all the presumptive diagnoses of sarcoidosis were correct, the incidence of positive liver biopsies was 75 per cent. This does not differ significantly from the incidence of sarcoidal lesions in the liver found at autopsy (63 per cent*), which suggests that the sampling error was insignificant, despite the small size of the tissue specimens obtained by needle biopsy.

The liver biopsy findings take on added significance when the results of other diagnostic measures are analyzed. The tuberculin test is usually given considerable weight in the differential diagnosis of sarcoidosis, although it is well known that it may be misleading. Only 6 of the 15 confirmed cases of sarcoidosis exhibited negative tuberculin reactions to the first and second strengths of P.P.D. (Table 3).

The x-ray findings in the chest frequently suggested or confirmed the clinical impression of sarcoidosis, but seldom warranted an unequivocal diagnosis. Intrathoracic adenopathy or pulmonary lesions compatible with sarcoidosis were observed in 13 of the 15 histologically-confirmed cases

* Based on autopsy reports of 74 cases taken from the literature.[1–16, 46–55] Most of the published autopsy reports on sarcoidosis were included, but a number were omitted because of inadequate data, the presence of extensive tuberculosis obscuring the distribution of sarcoidal lesions, or because the original reports were not available.

TABLE 5

DIFFERENTIAL DIAGNOSIS IN HISTOLOGICALLY-CONFIRMED SARCOIDOSIS

Case no.	*Roentgenologist's diagnoses (before biopsy)*	*Clinicians' diagnoses (before biopsy, after chest x-ray)*	*Initial biopsy establishing diagnosis*	*Later biopsies confirming diagnosis*	*Negative biopsies*
1	Bilateral pneumonia Probable tuberculosis Sarcoidosis (after splenectomy)	Banti's syndrome Tuberculosis Sarcoidosis (after splenectomy)	spleen*	liver lymph node skin	marrow
2	Hodgkin's disease ? Sarcoidosis*	Hodgkin's disease Tuberculosis Sarcoidosis	liver	0	lymph node
3	Pulmonary fibrosis ? Pneumoconiosis	Lymphosarcoma Hodgkin's disease Pulmonary fibrosis Banti's syndrome Sarcoidosis*	liver	0	marrow
4†	Probable Hodgkin's disease Minimal tuberculosis, L.U.L.	Hodgkin's disease Sarcoidosis* Tuberculosis	liver	0	0
5	Normal	Liver abscess Acute hepatitis Hodgkin's disease Banti's syndrome Malignancy Sickle anemia Sarcoidosis*	lymph node	liver	marrow
6	Probable Hodgkin's disease	Aortic aneurysm Hodgkin's disease Sarcoidosis* Tuberculosis	liver	0	0

7†	Sarcoidosis,* or Hodgkin's disease	Sarcoidosis Hodgkin's disease Coccidioidomycosis Tuberculosis	liver	0	0
8	Sarcoidosis ? tuberculosis ? lymphoblastoma	Sarcoidosis*	liver	lachrymal gland	0
9	? tuberculosis ? sarcoidosis*	Sarcoidosis	liver	0	lymph node
10†	? sarcoidosis* ? Hodgkin's disease	Sarcoidosis Hodgkin's disease	liver	0	marrow
11	? Hilar adenopathy	Pericarditis Collagen disease Lymphoma Tuberculosis Brucellosis	liver*	marrow	lymph node
12	Pulmonary fibrosis	Pulmonary fibrosis Hyperparathyroidism	lymph node*	liver	0
13	Normal	Hodgkin's disease Brucellosis Sarcoidosis*	liver	0	marrow
14†	? Sarcoidosis*	Lymphoma Sarcoidosis Infectious mononucleosis	liver	0	lymph node
15	Bronchopneumonia ? tuberculosis Sarcoidosis (later film)	Sarcoidosis*	liver	0	0

* First suggested diagnosis of sarcoidosis.

† Hospitalized for investigation of abnormal pulmonary findings discovered during routine x-ray examination.

(Table 2). In 7 of these, including 4 whose disease was discovered during a routine chest x-ray examination, the x-ray findings first called the attention of the clinician to the possibility of sarcoidosis. However, the clinician's interpretation of these findings was influenced by his knowledge of other clinical and laboratory features. A better appraisal of the relative value of the x-ray examination in the differential diagnosis of sarcoidosis can be obtained by analyzing the opinions rendered by expert roentgenologists who examined the initial films with a minimum of clinical data available. Sarcoidosis was mentioned as a possibility in only 6 of the 13 cases showing abnormalities, and in every instance but one an alternative diagnosis was mentioned (Table 5).

The x-ray findings in the chest were of relatively greater importance in the groups with a presumptive diagnosis of sarcoidosis. Roentgenological abnormalities compatible with sarcoidosis were the first clue to the nature of the diagnosis in all 5 cases. In 4 of these, sarcoidosis was suggested by the roentgenologist.

The x-ray findings in the extremities were of no value in this series, since not one of the 18 confirmed and presumptive cases examined exhibited the cystic areas occasionally seen in sarcoidosis.

The alterations in the protein pattern described by Harrell and Fisher[27] were observed in many of the subjects, although not as frequently as positive liver biopsies. Of the 15 histologically-proved cases, 7 had serum protein levels above 7.5, while 7 of the 12 tested had serum globulin levels above 3.5 grams per cent (Table 3). However, these changes proved to be of little value in the differential diagnosis of sarcoidosis because of their non-specificity.

The serum calcium concentration is frequently increased in sarcoidosis.[27] In this series only 1 of the 5 determinations carried out revealed significant hypercalcemia (12.89 mg. per cent) associated with mild hyperphosphatemia (5.16 mg. per cent). However, these findings proved to be of little diagnostic value in this subject (case 12). The patient was studied at another hospital where the discovery of hypercalcemia, hypercalcinuria, and bone decalcification associated with renal failure led to the diagnosis of hyperparathyroidism, and the performance of a parathyroidectomy. The correct diagnosis was not established until somewhat later when lymphadenopathy became apparent and a biopsy was performed.

Minor changes in the hematological picture were observed in a few subjects, but in none were they helpful in establishing the diagnosis of sarcoidosis (Table 3). Five subjects exhibited leukopenia, and one a mild monocytosis. Slight normochromic anemia occurred in 6 cases, and the sedimentation rate was increased in all but one of the 7 tested.

Histopathology of the liver. The histological features of the liver biopsy specimens obtained in sarcoidosis and in the other granulomatous diseases studied are outlined in Table 6. The outstanding finding in both groups was

the presence of multiple, discrete granulomata with minimal changes in the remaining parenchyma.

In general, the granulomata observed in the sarcoidosis group resembled those described by others, both in the liver[18, 19, 20] and in other tissues.[9, 11] However, the similarities between the hepatic lesions in sarcoidosis and the other granulomatous diseases studied proved to be much greater than anticipated from previous reports, and it was found that, with few exceptions, the lesions could not be differentiated on the basis of histological criteria alone. The striking similarity of the lesions in sarcoidosis, erythema nodosum, tuberculosis, and brucellosis is illustrated in Figures 1, 2, 3, and 4.

Judging by the ease with which granulomata were demonstrated by needle biopsy, and from the *number* of lesions in single sections, ranging from 1 to more than 12, it was apparent that the livers in the subjects with sarcoidosis were heavily seeded with granulomata. Similar numbers of lesions were found in the other granulomatous diseases studied, although the incidence of positive liver biopsies was significantly lower in some forms of tuberculosis (Table 1).

The *size* of the granulomata tended to remain submiliary in all groups, but they were by no means uniform. The largest lesions comprised collections of 25 to 50 epithelioid cells; the smallest, of less than 12. In most subjects both small and large lesions could be found in the same section, and usually the small lesions outnumbered the large.

The *contour* of the lesions in sarcoidosis tended to be round and discrete with sharp borders (Fig. 1B), while that in tuberculosis and brucellosis was more often irregular or lobulated, suggesting the coalescence of multiple lesions (Figs. 2D, 4B). However, lobulated or irregular lesions were also observed in some cases of sarcoidosis (Fig. 1D), and round, sharply outlined lesions in some cases of tuberculosis (Fig. 1C). Moreover, the contour of the granulomata often varied in the same section, so that this criterion was of little value in differentiating hepatic granulomata. Others[56] have emphasized as a point of differentiation the tendency of tuberculous lesions to coalesce.

The *localization* of the lesions in sarcoidosis did not differ significantly from that in tuberculosis and brucellosis. Granulomata were found both within the parenchyma and in the portal zones in all three diseases. The lesions in erythema nodosum and in the virus and mycosis groups all occurred within the lobular parenchyma. However, differences in localization should not be overemphasized, especially when based on the examination of single sections. In many instances, lesions which were clearly intralobular in one section proved to have connections with a portal zone when traced through serial sections, and even then it was not always possible to determine the site of origin of such lesions. Previous reports[11, 40]

TABLE 6

SUMMARY OF HISTOLOGICAL FEATURES IN LIVER BIOPSY MATERIAL

Case no.	*Hepatic granulomata*													*Associated changes*							
											Giant cells			*Portal zones*			*Parenchyma*				
	No. lesions/ section	*Size*	*Location*	*Shape*	*Border*	*Collagen envel.*	*Central reticulum*	*Density*	*Cellular infil.*	*Cellular border*	*No. per section*	*Inclusions*	*Necrosis*	*Connective t. prolif.*	*Cellular infiltration*	*Bile duct proliferation*	*Fatty infiltration*	*Necrosis*	*Cellular infiltration*	*Focal R.E. hyperplasia*	*Clinical diagnosis*
1	3	L, S	P, H	round	sharp	+	+	+	0	1	3	0	0	+	0	+	0	+	l, e	+	sarcoidosis
2	12	L, S	P, H	lobul.	sharp	+	+	++	l, e	e, p	0	—	+f	0	l, e, p	0	0	0	e	+	sarcoidosis
3	8	L, S	P, H	lobul.	sharp	+	—	+++	0	1	5	+c	0	0	0	0	+	0	0	+	sarcoidosis
4	3	L, S	H	irreg.	irreg.	0	—	++	1	1	0	—	0	0	0	0	+	0	0	+	sarcoidosis
5	>12	L, S	P, H	round	sharp	0	+	+++	0	p	0	—	0	+	0	0	0	0	0	+	sarcoidosis
6	10	L, S	H	irreg.	irreg.	0	+	++	0	0	1	0	0	0	0	0	0	0	0	+	sarcoidosis
7	16	L, S	P. H	lobul.	sharp	0	+	+++	0	0	3	0	0	0	0	0	+	0	0	+	sarcoidosis
8	2	S	P, H	round	sharp	0	—	+	0	0	0	—	0	0	0	0	0	0	0	+	sarcoidosis
9	3	S	P, H	round	sharp	+	—	+	0	0	6	0	0	0	0	0	0	0	0	0	sarcoidosis
10	2	L, S	P. H	irreg.	irreg.	0	—	+++	0	1	2	0	+f	0	0	0	+	0	0	+	sarcoidosis
11	6	M	P, H	round	sharp	++	—	+	p, l, e	p, l, e	6	+v	0	+	p, l, e	0	0	+	0	0	sarcoidosis
12	4	L, S	P, H	irreg. round	irreg. sharp	0 +	+	+++	1	1	4	+c	+	0	0	0	+	0	0	+	sarcoidosis
13	< 1	S	P	round	irreg.	0	+	++	0	0	0	0	0	+	0	+	0	0	0	0	sarcoidosis
14	1	S	H	irreg.	irreg.	0	—	++	0	l, m	0	0	0	0	0	0	0	0	0	0	sarcoidosis
15	< 1	S	H	round	sharp	0	—	++	0	0	0	—	0	0	0	0	0	0	0	0	sarcoidosis

16	3	L	H	round	sharp	+	—	++	0	0	3	0	0	0	0	0	0	0	0	miliary tuberculosis
17	4	L, S	H	round irreg.	sharp irreg.	0	±	++	l, m	1	0	—	+	0	1	0	0	+	l, m	+	miliary tuberculosis
18	2	L, S	P	irreg.	irreg.	0	—	+++	0	1	5	0	+++c	0	0	0	0	0	0	0	miliary tuberculosis
19	3	L	P, H	irreg.	irreg.	0	—	+	0	1	2	0	+++	0	1	0	0	0	0	0	miliary tuberculosis
20	6	S	P, H	irreg.	irreg.	0	0	++	1	0	0	0	+f	0	0	0	0	0	0	+	pulmon. tuberculosis
21	1	M	P	lobul.	irreg.	0	—	++	0	0	1	0	+++	0	0	0	+	0	0	0	pulmon. tuberculosis
22	1	M	P, H	lobul.	sharp	+	—	+++	0	1	0	—	0	0	0	0	0	0	0	0	tuberculin-neg., erythema nodosum
23	2	L, S	P, H	irreg. lobul.	irreg. sharp	0	+	++	1	1	3	0	+	0	0	0	0	0	0	0	tuberculin-pos., erythema nodosum
24	7	L, S	P, H	irreg. lobul.	irreg. sharp	0	—	++	1	1	3	0	+++	++	l, pl.	0	+	0	l, pl.	+	brucellosis
25	2	S	H	irreg.	irreg.	0	—	++	0	0	0	—	0	0	l, m	l, m	0	+	l, m	++	infect. mononucleosis
26	4	L, S	H	irreg.	irreg.	0	—	++	1	1	0	—	+	0	0	0	0	0	0	0	influenza B
27	2	M, S	H	irreg.	irreg.	0	—	++	0	0	0	—	+	+	p, l, pl, m	0	+	+	m	+	fever, ? viral
28	1	S	H	irreg.	irreg.	0	—	++	1	1	0	—	+	0	1	0	0	0	0	+	actinomycosis
29	10	L, S	P, H	round	sharp	0	—	+	0	0	5	0	0	+	+	0	0	0	0	+	inf. hepatitis
30	1	L	P, H	round	sharp	+	—	++	0	l, e	3	+a	0	+++	0	0	++	0	0	0	cirrhosis, diabetes
31	>12	L	P, H	lobul.	sharp	+	—	+	l, e	l, e	>12	++v	+	++	l, e	++	0	0	0	0	cirrhosis
32	2	S, M	P, H	irreg.	irreg.	0	—	++	0	1	1	0	0	++	m	0	+	+	1	0	cirrhosis

Symbols: Size: L = >24 epithelioid cells, M = 12-24 cells, S = <12 cells. Location: P = portal, H = intralobular. Density: + = loose, ++ = moderately compact, +++ = compact. Cell types: p = polymorphonuclears, l = lymphocytes, e = eosinophiles, m = monocytes, pl = plasma cells. Necrosis: f = fibrinoid, c = caseous. Inclusions: c = crystalline, a = asteroids, v = vacuoles.
+ = mild, ++ = moderate, +++ = marked.

have emphasized the predominance of sarcoidal lesions in the portal zones and tuberculous lesions within the lobular parenchyma.[11, 40]

The lesions in sarcoidosis were composed of masses of *epithelioid* cells, varying in shape, size, and arrangement, depending in part on the compactness of the lesion. Neither the histological appearance of these cells individually, nor their arrangement or compactness, differed from those found in the other granulomatous diseases studied. The staining properties of the epithelioid cells differed very markedly from those of hepatic parenchymal or other inflammatory cells, and were especially well brought out by the Masson trichrome staining technique. In sections so stained even the smallest lesions stood out in bold relief against the background of normal liver tissue.

The paucity of *inflammatory cells* within and around the granulomata in sarcoidosis has been emphasized as a point of differentiation from other granulomata. In general, the lesions in tuberculosis and brucellosis did show a more dense collar of lymphocytes than those in sarcoidosis, in which disease such cells were often lacking, but there were too many exceptions in both groups to validate this as a reliable criterion for histological differentiation. The distribution of lymphocytes and other leukocytes within the granulomata was of no greater value, since they occurred only infrequently and in small numbers in all the diseases studied.

Giant cells were demonstrated in 8 of the 15 cases of sarcoidosis, in 5 of the 7 cases of tuberculosis, in one of the 2 cases of erythema nodosum and in the one case of brucellosis studied, but neither their number nor type served to differentiate between these diseases. Typical Langhans' and foreign body giant cells could be found in each of the groups, and often within the same lesion when studied in serial sections. Giant cells of unusual size, and containing an unusually large number of nuclei, are said to be characteristic of sarcoidosis.[9, 40] In the present series neither the size nor the nuclear content of the giant cells differed significantly in the various disease groups studied.

Giant-cell inclusions of the asteroid[9] and Schaumann-body type[37] are frequently found in the granulomata of sarcoidosis, although they are by no means pathognomonic.[25] Only 2 of the histologically-confirmed cases of sarcoidosis exhibited crystalline inclusions, possibly an early stage of the asteroid body, and case 30 with cirrhosis and a presumptive coincidental sarcoidosis had a typical asteroid body. No Schaumann bodies were observed, but it is known that these occur more frequently in the lymph nodes than in other tissues.[37] Teilum[58] has suggested that the inclusion bodies in sarcoidosis represent globulin precipitates related to hyperglobulinemia. In this series there was no correlation between the occurrence of inclusion bodies and the concentration of serum globulin. No inclusion bodies were observed in tuberculosis or brucellosis. Although inclusions do

occur in granulomatous diseases other than sarcoidosis, they rarely occur in tuberculosis.[25]

The presence of a *reticulum* framework in the granulomata of sarcoidosis is regarded as an important point in its differentiation from tuberculosis.[11] A fine reticulum network was demonstrated in each of the 6 cases of sarcoidosis examined. In 2 subjects with tuberculosis no reticulum was demonstrated in one, and only a few scattered fibers in the other. In subject 23, a case of tuberculin positive erythema nodosum, the hepatic granulomata were thought to be tuberculous, but they exhibited a reticulum framework as in sarcoidosis.

There was a striking difference between the degree of *necrosis* observed in the granulomata of sarcoidosis, tuberculosis, and brucellosis. Necrosis was absent in 12 and was only minor in extent in 3 of the 15 cases of sarcoidosis. In 7 cases of tuberculosis, on the other hand, all but one showed necrosis, and it was frequently extensive. Necrosis was also extensive in the one case of brucellosis. It is of interest that caseation necrosis occurred in only one of the 7 cases of tuberculosis, a patient who had had signs of miliary tuberculosis for 6 months (Fig. 2D). Although the demonstration of marked necrosis favored either tuberculosis or brucellosis, its absence could not be used to differentiate these diseases from sarcoidosis. In 3 of the tuberculous subjects the foci of necrosis were very small, and could not be differentiated from those seen in some cases of sarcoidosis, and in all the tuberculous subjects and in the one with brucellosis, there were small granulomata without necrosis adjacent to the larger necrotic lesions. One subject with miliary tuberculosis exhibited granulomata with no necrosis whatever.

A thin *collagen envelope* was demonstrated in some of the lesions in 6 cases of sarcoidosis. In one subject (case 11) the envelope was laminated, suggesting the amorphous paramyloid bands described by Teilum.[54] Sarcoid lesions are known to undergo fibrosis and hyalinization during the stage of healing,[11] but in the present series there did not appear to be any relationship between the presence of collagenous connective tissue encapsulation and the known duration of the disease. Similar encapsulation was observed in one case of tuberculosis and in a case of tuberculin-negative erythema nodosum, but not in brucellosis.

Focal areas of intrasinusoidal *reticulo-endothelial hyperplasia* were common in both sarcoidosis and other granulomatous diseases. In some sections it was possible to demonstrate intermediate stages between these ill-defined, irregular collections of swollen Kupffer cells and sharply outlined, small epithelioid granulomata, suggesting that the former were in some instances, at least, the forerunners of the latter.

Minor associated changes in the liver, unrelated to the granulomatous lesions themselves, were observed in many of the confirmed cases of sarcoidosis. These consisted of mild degrees of periportal fibrosis and

cellular infiltration, and less commonly of mild fatty infiltration, focal necrosis or cellular infiltration of the parenchyma. In none did these changes suggest an early cirrhosis. The relationship of sarcoid-like hepatic lesions to the development of cirrhosis in 3 subjects (cases 30, 31, and 32) will be discussed in another section.

A much greater degree of connective tissue proliferation and cellular infiltration of the portal zones and of cellular necrosis and inflammatory reaction in the parenchyma was observed in brucellosis. The possible relationship of these lesions to the development of cirrhosis will be discussed in another section.

No significant alterations in hepatic structure, other than the presence of granulomata, were observed in tuberculosis.

Acid-fast stains for tubercle bacilli were uniformly negative, except in one of the 4 subjects with miliary tuberculosis who exhibited typical tubercle bacilli adjacent to a granuloma.

Sarcoid-like hepatic lesions associated with other diseases

The striking similarities between the hepatic lesions of sarcoidosis and those of other granulomatous diseases may be paralleled by indistinguishable clinical features, a fact which emphasizes the necessity for carefully considering all the granulomata in any study of sarcoidosis, whether it be from the point of view of the clinician interested in diagnosis, or from that of the investigator concerned with its pathogenesis.

The following is a résumé of the clinical and laboratory evidence of liver involvement in the more common granulomatous diseases encountered during this investigation. The data illustrate the difficulties which these diseases may present in the differential diagnosis of sarcoidosis, and demonstrate some interesting relationships which may be of significance in the pathogenesis of sarcoidosis.

Acute miliary tuberculosis. The liver is frequently seeded with tubercles during the course of acute miliary tuberculosis, and in occasional cases the seeding may be so dense that the clinical picture is dominated by jaundice and hepatomegaly. As might be expected, therefore, needle biopsy of the liver has proved to be of diagnostic value in this disease.[59, 60]

Four subjects with acute miliary tuberculosis were investigated. The clinical features of the disease are summarized in Table 7. The symptoms had an acute onset and were of relatively brief duration in cases 16 and 17, while the clinical course was less acute and more protracted in cases 18 and 19. Hepatomegaly was evident only in the more chronic form of the disease, but hepatic granulomata and abnormalities of liver function were demonstrated in all four cases. In case 16 mild jaundice appeared on the third day of streptomycin therapy and subsided within a period of two weeks. The jaundice was associated with an increase in serum bilirubin,

TABLE 7

SUMMARY OF FINDINGS IN FOUR CASES OF DISSEMINATED MILIARY TUBERCULOSIS

	Case 16							*Case 17*	*Case 18*	*Case 19*
Age, sex, race	43, F, W							45, M, N	42, F, N	28, M, N
Symptoms and signs										
duration	18 days							10 days	6 months	3 months
pulmonary (x-ray)	+							0 (+ later)	0	+
pleural effusion	+							0	+	0
pericardial effusion	0							0	+	0
ascites	0							0	0	0
hepatomegaly	0							0	+	+
splenomegaly	0							0	+	0
jaundice	+							0	0	0
meningitis	later							0	+	+
lymphadenopathy	0							±	±	0
Tuberculin test	neg. (later +)							+	—	—
Serum proteins, gm.%	7.16							6.93	7.08	7.20
serum albumin	2.60							2.38	3.13	—
serum globulin	4.56							4.55	3.95	—
Liver function tests	*7/18*	*7/20*	*7/26*	*8/1*	*8/17*	*9/19*	*10/11*			
ser. l'bilirubin, mg.%	1.44	—	0.48	0.50	0.14	0.22	0.07	0.05	0.05	0.30
ser. total bilirubin, mg.%	2.66	—	1.18	1.32	0.66	0.28	0.47	0.80	0.58	1.50
ceph.-chol. flocc.	—	4+	4+	4+	4+	3+	0	4+	3+	4+
thymol turbidity, u.	—	14.5	16.0	13.5	14.0	9.0	7.5	11.0	16.2	2.5
ser, alk. phosph., B. u	—	40.4	25.8	—	35.3	26.1	14.1	9.37	2.9	—
urine urobilinogen	—	—	—	—	—	—	—	1.80	—	—
BSP retention, % in 45′	—	44.1	—	16.5	—	14.7	12.3	32.7	7.5	2.5
Tubercle bacilli										
sputum	0 (gu. pig)							+ (gu. pig)	—	0
gastric washings	+ (gu. pig)							—	—	0
spinal fluid	0 (smear)							—	0 (smear)	+ (smear)
Liver biopsy	+							+	+	+
Diagnosis established	Chest x-ray							Liver biopsy	Liver biopsy	Chest x-ray Spinal fluid

strongly positive cephalin-cholesterol and thymol turbidity tests, and a striking elevation of serum alkaline phosphatase. The pathogenesis of the jaundice was not clear. Unfortunately, the liver biopsy specimen, which revealed many granulomata, was not obtained until the jaundice had subsided. The relatively short duration of the jaundice (despite the continued presence of many tubercles), its close association with the commencement of streptomycin therapy, and the unusually high serum alkaline phosphatase level, suggested the possibility of a drug reaction of the type seen in early arseno-therapy.[61] Tuberculosis can, of course, involve the biliary tree[62] and produce a similar picture, but this mechanism seemed unlikely in view of the brief duration of the jaundice. In the other cases, the laboratory evidence of liver damage was less striking, and the strongly positive cephalin-cholesterol flocculation and thymol turbidity reactions observed may have been more closely related to the hyperglobulinemia associated with tuberculous infection than to specific hepatic lesions.

The diagnosis of miliary tuberculosis was based on the liver biopsy findings in two of the four cases. In case 17 tubercles and acid-fast bacilli were demonstrated in the liver a week before miliary lesions were evident in the chest x-ray (5th week of the disease), and a month before acid-fast bacilli were demonstrated by guinea-pig inoculation of a sputum concentrate. Likewise in case 18, the diagnosis was based on the finding of typical caseating tubercles in the liver. Acid-fast bacilli were never demonstrated in stained smears or cerebrospinal, pleural, or pericardial exudate, and pulmonary lesions did not become evident by x-ray before death. Unfortunately sputum and gastric washings were not obtainable because of the patient's critical state.

In cases 16 and 19 the clinical diagnosis was based on the x-ray findings in the chest. Later, tubercles were demonstrated in the liver, but they contained no acid-fast bacilli and exhibited no caseation necrosis. They were compatible with, but not diagnostic of, tuberculosis. Confirmation of the diagnosis depended on the recovery of tubercle bacilli from exudates in both cases.

These results illustrate both the value and the limitations of needle biopsy of the liver in the diagnosis of miliary tuberculosis. The early acute phase of the disease, especially before the appearance of pulmonary lesions or meningitis, may simulate a wide variety of febrile diseases. The demonstration of hepatic granulomata narrows the range of possibilities to the granulomatosis group, but does not exclude such diseases as brucellosis or the more florid forms of sarcoidosis, unless tubercle bacilli or caseation necrosis can be demonstrated in the sections. Often the granulomata are not distinctive and the diagnosis of miliary tuberculosis cannot be made without collateral bacteriological or clinical evidence.

Other forms of tuberculosis. Miliary tubercles are found with great regularity in the livers of fatal cases of chronic tuberculosis. In Saphir's

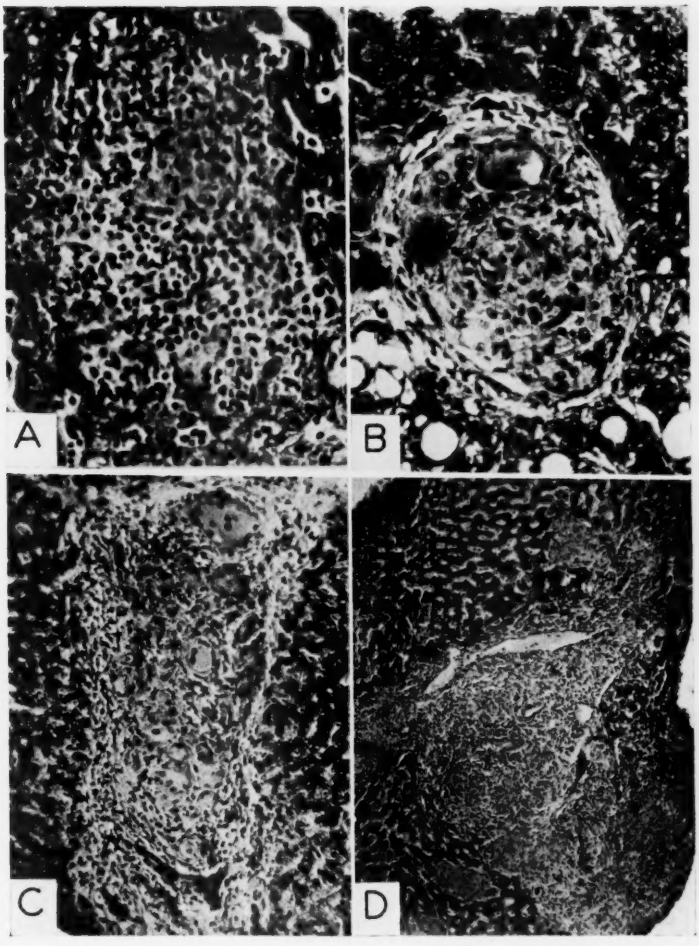

FIG. 1. Hepatic granulomata in sarcoidosis. A. Case 4. Irregular intralobular lesions with poorly defined margins and considerable lymphocytic infiltration (x440). B. Case 12. Sharply circumscribed intralobular lesion with connective tissue capsule. Note giant cell with large vacuole containing crystalline material (x440). C. Case 3. Sharply circumscribed portal zone lesion with connective tissue capsule (x220). D. Case 2. Large lobulated portal zone lesion (x125).

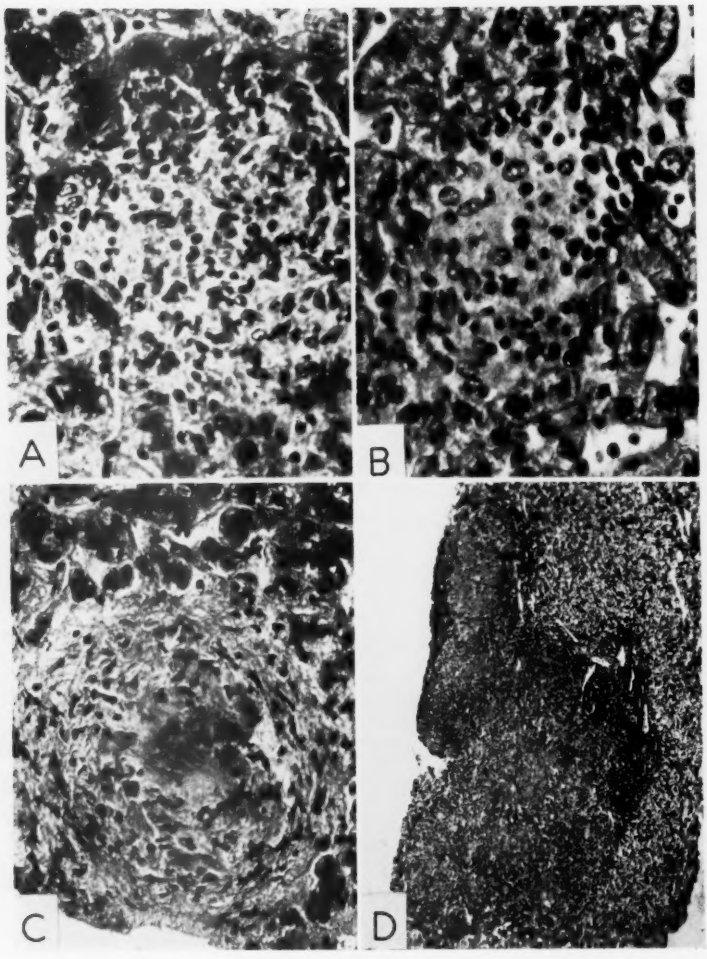

FIG. 2. Hepatic granulomata in tuberculosis. A. Miliary tuberculosis (case 17). Irregular intralobular lesion with moderate lymphocytic and monocytic infiltration. Acid-fast stain revealed tubercle bacilli adjacent to a similar lesion (x550). B. Pulmonary tuberculosis (case 20). Small, poorly circumscribed intralobular lesion with slight lymphocytic infiltration (x750). C. Miliary tuberculosis (case 16). Sharply circumscribed intralobular lesion with thick connective tissue capsule and little inflammatary exudate (x440). D. Miliary tuberculosis (case 18). Large irregular portal zone lesion with dense surrounding lymphocytic infiltration and areas of central caseation necrosis (x85).

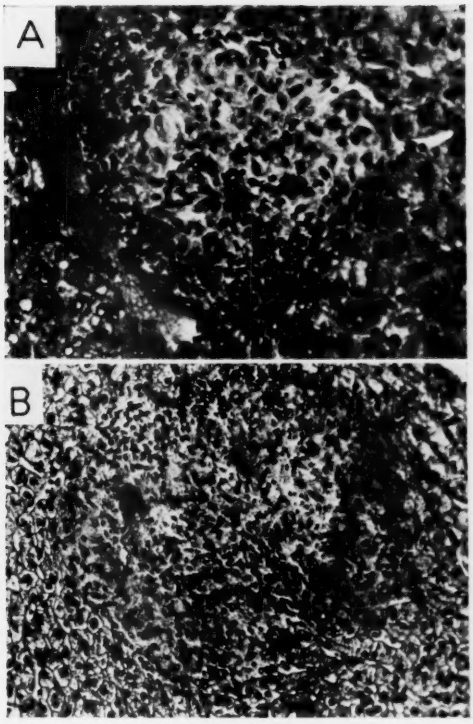

FIG. 3. Hepatic granulomata in erythema nodosum. A. Tuberculin-negative erythema nodosum (case *22*). Sharply circumscribed lesion adjacent to a small sublobular vein showing slight surrounding lymphocytic reaction (x440). B. Tuberculin-positive erythema nodosum associated with active tuberculosis of the spine (case 23). Lobulated intralobular lesion with poorly defined borders, moderate lymphocytic reaction, and slight central necrosis. Central reticulum network demonstrated by special stain (x250).

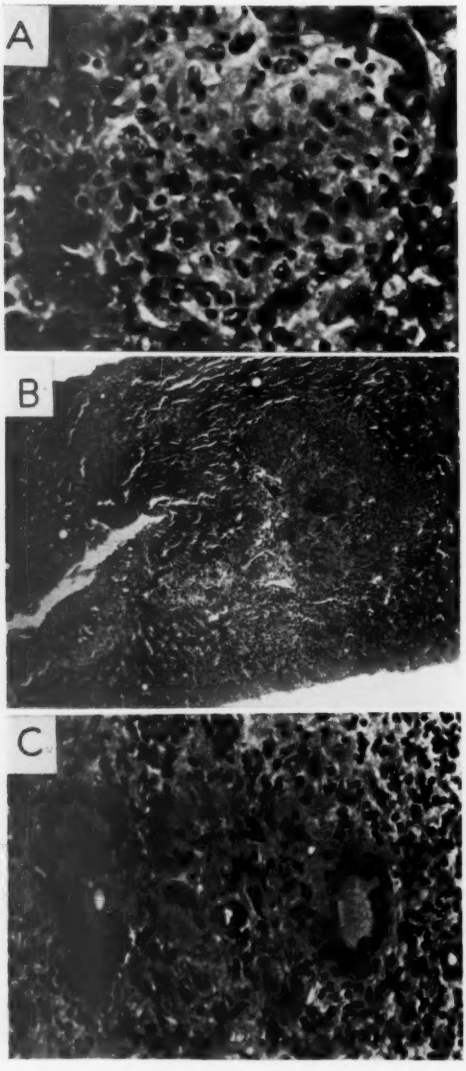

FIG. 4. Hepatic granulomata in brucellosis (case 24). A. Sharply circumscribed lobulated intralobular lesion with slight lymphocytic inflammation (x650). B. Very large irregular portal zone lesion extending to a central vein and along the wall of a sublobular vein. Marked lymphocytic infiltration and small areas of central necrosis (x110). C. High power view of Fig. 4B, showing giant cells with many nuclei, epithelioid cells, and lymphocytic infiltration (x440).

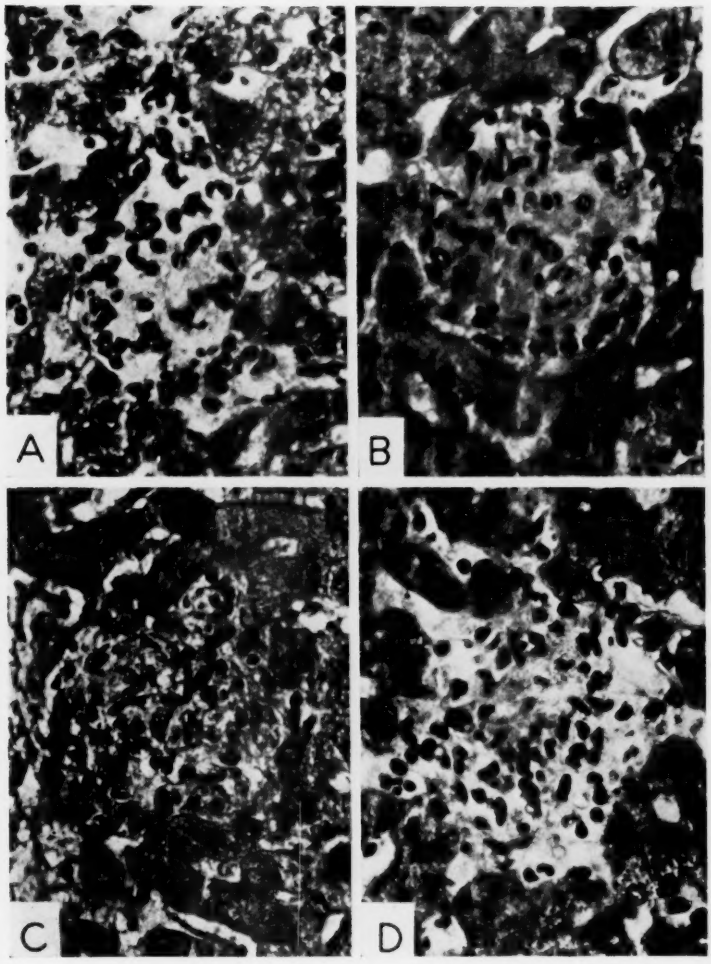

FIG. 5. Small atypical hepatic granulomata associated with infection. A. Infectious mononucleosis (case 25) x740. B. Influenza B (case 26) x750. C. Disseminated actinomycosis (case 28) x720. D. Viral infection, type not determined, (case 27) x700.

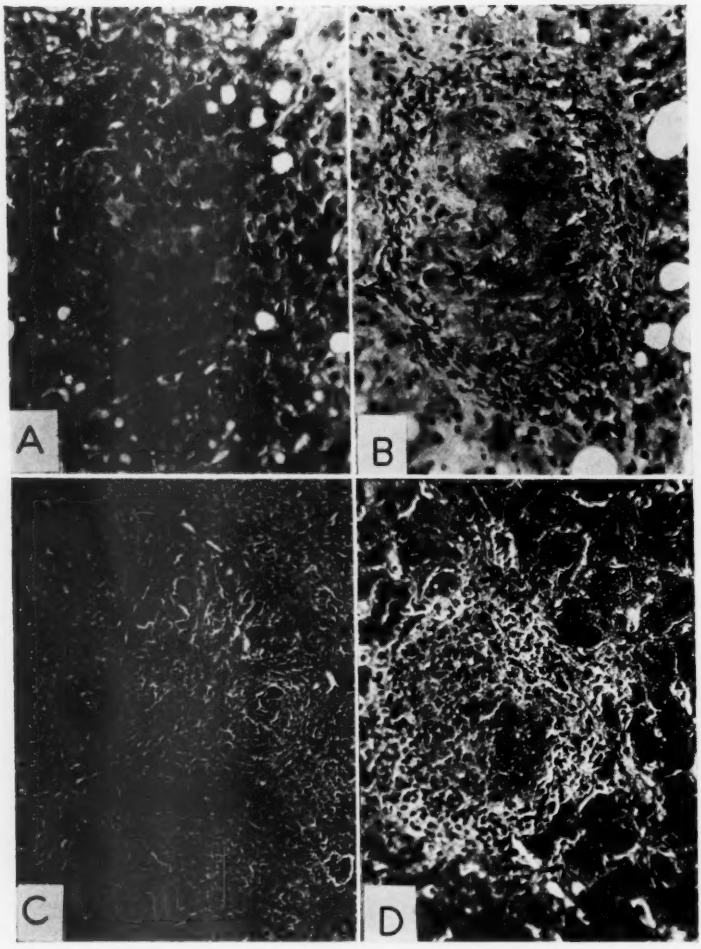

FIG. 6. Hepatic granulomata in subjects with "primary" liver disease. A. Infectious hepatitis (case 29). Intralobular lesion interpreted as evidence of coincidental sarcoidosis (x750). B. Cirrhosis (case 30). Intralobular lesion interpreted as evidence of coincidental sarcoidosis (x300). C. Granulomatous cirrhosis (case 31). Extensive granulomatous infiltration of portal zones and parenchyma distorting the normal lobular architecture, interpreted as evidence of sarcoidal cirrhosis (x140). D. Cirrhosis (case 32). Intralobular lesion with considerable surrounding lymphocytic reaction (x275). Nature of lesion not determined.

series[63] they occurred in 80 out of 100 cases of chronic ulcerative pulmonary tuberculosis. The hepatic tubercles did not appear to be related to the presence of intestinal lesions, suggesting that hematogenous spread was by way of the hepatic artery. Lorentz[64] reviewed the autopsy findings in 100 cases of chronic tuberculosis, including 14 with major lesions in extrapulmonary sites, and found that all but one had tubercles in the liver. In this series there was a very close relationship between the occurrence of hepatic and intestinal lesions, implying an hematogenous spread of tubercle bacilli by way of the portal vein. In the light of this experience Lorentz concluded that such hematogenous spread occurred in every case of florid tuberculosis. The clinical significance of these observations has received little attention until recently. Clinicians have long recognized the manifestations of overwhelming hematogenous dissemination of tubercle bacilli, but have not been able to detect the minor episodes that occur during the course of chronic tuberculosis, unless they have resulted in obvious disease of such tissues as the lung, genito-urinary tract, or bone. Recently, however, van Beek and Haex[59] have been able by means of liver biopsy to demonstrate hematogenous dissemination in all types of chronic tuberculosis, not only of the lungs, but also of the glands and viscera. Their findings are more in keeping with autopsy experience than those of van Buchem,[19] who could find no hepatic granulomata in the 9 cases of active exudative pulmonary tuberculosis he biopsied. Van Beek and Haex[59] stressed the importance of sectioning biopsy specimens serially in searching for hepatic tubercles. Van Buchem[19] does not appear to have used this technique, which may account for his results. Recognition of the fact that miliary tubercles, sometimes indistinguishable from sarcoids, may occur in the liver with pulmonary or grandular tuberculosis is of the greatest importance in interpreting the liver biopsy findings in suspected sarcoidosis.

Liver biopsies were performed in 13 cases of active tuberculosis. The disease was predominantly pulmonary in 8, glandular in 3, renal in 1, and osseous in 2. All blocks of tissue were sectioned serially, and in 5 cases the biopsy was repeated. Miliary granulomata were demonstrated in 3 of the 13 cases. Case 23, in which osseous tuberculosis was complicated by tuberculin-positive erythema nodosum, will be described in the section on erythema nodosum. A brief summary of the findings in the remaining two cases follows.

Case 20. W. Mc., a 35-year-old Negro, was found to have bilateral pulmonary infiltration, a questionable cavity in the left upper lobe, and marked hilar adenopathy in May 1949 during a routine community x-ray survey. These changes were interpreted as indicating far advanced pulmonary tuberculosis. An x-ray of the chest taken in 1943 was normal. He had no symptoms except for mild cough of several weeks' duration, attributed to a recent upper respiratory infection.

Physical examination revealed nothing of note except for pulmonary findings compatible with bilateral tuberculosis.

The patient was admitted to a tuberculosis sanatorium in September 1949 and

remained there until April 1950. During that period he was asymptomatic; serial x-rays of his chest revealed no change; monthly sputum concentrates were negative for acid-fast bacilli on smear and culture, and one gastric-washing was negative for acid-fast bacilli on culture and guinea-pig inoculation. Tuberculin tests with first and second strengths of PPD were both negative. In April 1950 the patient developed a prolonged, unexplained fever, and three weeks later was transferred to this hospital for further investigation.

Physical examination revealed mild generalized lymphadenopathy, pulmonary findings as before, and fever.

Repeated blood cultures and brucella agglutination tests were negative. Hyperglobulinemia was present (total proteins 7.44, albumin 3.16, globulin 4.28 mg. per cent). Liver function tests revealed the following: one-minute serum bilirubin 0.07 mg. per cent, total serum bilirubin 0.52 mg. per cent, cephalin-cholesterol focculation 3+, thymol turbidity 10 units, serum alkaline phosphatase 6.4 Bodansky units, bromsulphthalein 5.4 per cent retention in 45 minutes, and prothrombin 74 per cent. X-ray examination of the chest showed no significant change since the initial examination in April 1949, and once again was interpreted as indicative of bilateral pulmonary tuberculosis, although the diagnosis of sarcoidosis was suggested as a possibility. No changes in the bones were demonstrated by x-ray.

Liver biopsy revealed the presence of granulomata, consisting of epithelioid cells without giant cells (Fig. 2B). The lesions were regarded as compatible although not typical of sarcoidosis, since many were poorly circumscribed and lacked a reticulum network. Acid-fast stain revealed no bacilli. Biopsy of an axillary lymph node revealed large masses of epithelioid cells surrounded, in some areas, by zones of eosinophilic amorphous material, changes which were interpreted as indicative of "probable Boeck's sarcoid," although the pathologist indicated that tuberculosis could not be excluded. Unfortunately, none of the gland was saved for guinea-pig inoculation.

The patient was discharged from the hospital on April 29, 1950, with the diagnosis of sarcoidosis. He was still mildly febrile but felt well. His clinical improvement continued as an ambulatory patient. In June 1950 the guinea-pig inoculated with gastric washings obtained on April 28 was found to be positive for tuberculosis.

Comment. This case illustrates the difficulty of differentiating sarcoidosis from tuberculosis. The diagnosis of sarcoidosis was based on the histological findings in both the liver and a lymph node, the relatively benign course of the disease, a negative tuberculin reaction, hyperglobulinemia, the absence of tubercle bacilli in the sputum, and the stability of the pulmonary lesions over a long period of observation. Until tubercle bacilli were demonstrated by guinea-pig inoculation of gastric washings, it was assumed that the pulmonary lesions were due to sarcoidosis. In retrospect, it seems probable that they were tuberculous in nature, and that the acute febrile illness represented a hematogenous spread. The granulomata in the liver were composed largely of epithelioid cells, the type of lesion described by van Beek and Haex[39] in early hematogenous dissemination. They, too, failed to observe acid-fast bacilli or caseation under such conditions. It is, of course, possible to take the point of view that the patient had two unrelated diseases, sarcoidosis and tuberculosis, or, as suggested by Scadding[42], that this was an example of tuberculous sarcoidosis, but the weight of evidence in the light of van Beek and Haex's work favors a tuberculous etiology for all the lesions.

Case 21. J.P., a 34-year-old white male, was admitted to the hospital on March 20, 1950, complaining of dysphagia, sore throat, and hoarseness of five weeks' duration. For one year he had noted a productive cough, increased fatigability, and moderate weight loss. Night sweats appeared two weeks before admission.

Physical examination revealed moderately enlarged, firm, non-tender submaxillary and axillary lymph nodes, signs of cavitation over the right upper lobe of the lungs and a pleuro-pericardial friction rub over the left anterior chest. The liver and spleen were not palpable. There were no skin or ocular lesions and the parotids were not enlarged.

X-ray examination of the chest revealed advanced exudative cavernous tuberculosis of the right upper lobe and minimal tuberculosis of the left upper lobe. A concentrate of sputum was positive for acid-fast bacilli on stained smear. The tuberculin test was strongly positive and the sedimentation rate was increased. Liver function tests and the serum protein concentrations were normal.

Liver biopsy revealed a typical granuloma with irregular margins in a portal zone. It showed only slight lymphocytic reaction around it, but exhibited considerable non-caseous central necrosis and contained a single giant cell. No acid-fast bacilli were demonstrable.

On supportive therapy plus streptomycin and tibione, there was prompt symptomatic improvement and the pleuro-pericardial friction rub and hoarseness disappeared. The temperature was slightly elevated for two days, but did not exceed 100°F. thereafter. The patient has been followed for three months and is still under observation. He continues to improve and has shown no clinical evidence of hematogenous dissemination. Liver biopsy repeated on April 3, 1950, failed to disclose tubercles, but the specimen obtained was very small.

Comment. This is another example of a clinically unrecognized hematogenous spread of tubercle bacilli to the liver during the course of active pulmonary tuberculosis. The granulomata in the liver were fewer in number than in case 20, but they were larger, were confined to the portal triads, and showed more necrosis, features indicating greater age, according to van Beek and Haex.[89] Although no tubercle bacilli were demonstrated in these lesions, their irregular contour and the degree of necrosis they exhibited were compatible with a tuberculous etiology. The exclusion of other necrotizing granulomata, such as brucellosis, however, was based on the accompanying clinical and bacteriological features, rather than on histological criteria.

Erythema nodosum. It is generally believed that erythema nodosum is a manifestation of hypersensitivity to a variety of agents, including bacteria, viruses, fungi, and chemical compounds.[28, 65] In an exhaustive investigation carried out in Sweden, Löfgren[66] found evidence of a tuberculous etiology in the majority of his cases. However, in a similarly large series studied in Boston by Favour and Sosman,[65] the beta-hemolytic streptococcus appeared to be the most frequent etiological factor.

The typical skin manifestations of erythema nodosum are occasionally accompanied by disseminated lesions involving the hilar lymph nodes, the lungs, the peripheral lymph nodes, and, more rarely, the eyes.[97] The nature of these lesions has aroused great interest. Löfgren[66] believes that the hilar

adenopathy is due to tuberculosis in tuberculin-positive cases, but that disseminated lesions in tuberculin-negative cases are probably manifestations of sarcoidosis. Kerley[67] attaches no importance to the results of the tuberculin reaction and asserts that all cases of erythema nodosum with visceral lesions are due to sarcoidosis.

The recent observations of van Beek and Haex[28] have been cited in support of the hypothesis that hilar adenopathy in tuberculin-positive erythema nodosum is due to tuberculosis. These investigators found, on liver biopsy in such cases, granulomata which resembled the hepatic tubercles seen in miliary tuberculosis. Although they were unable to demonstrate acid-fast bacilli in the lesions, they interpreted their findings as evidence of recent hematogenous dissemination of tubercle bacilli. They attached little importance to their failure to demonstrate organisms in hepatic tissue, since the same difficulty was encountered in the case of miliary tuberculosis. However, their conclusions regarding the tuberculous nature of the lesions, based on histological criteria and supported only by a positive tuberculin reaction and, in some cases, by evidence of tuberculosis in other tissues, cannot be accepted without reservation, in view of the difficulty of distinguishing between tuberculosis and other types of hepatic granuloma on histological grounds alone. Moreover, the authors presented no data on the hepatic findings in tuberculin-negative erythema nodosum. Whether tuberculous and non-tuberculous types of erythema nodosum can be differentiated on the basis of the tuberculin reaction is debatable, but it is clear that the presence of hepatic granulomata cannot serve to distinguish between them.

The interrelationships between erythema nodosum, sarcoidosis, and tuberculosis are poorly understood. There are several striking similarities between erythema nodosum and sarcoidosis. They are both regarded, by some at least, as a non-specific response to a number of etiological agents, both may exhibit disseminated lesions of similar distributions and histological structure, and both have been considered as a special type of response to the tubercle bacillus in some cases. The evidence suggests that both sarcoidosis, or a sarcoid-like syndrome, and erythema nodosum may be produced by a variety of etiological agents, including the tubercle bacillus, and that in some cases the same agent may produce both diseases simultaneously.

During the course of this study, 3 cases of erythema nodosum were investigated. In 2 of the 3 cases, granulomata resembling sarcoids were demonstrated on liver biopsy. One of the cases was tuberculin-negative and had no evidence of tuberculosis, the other was tuberculin-positive and had Pott's disease and a pleural effusion, presumably of tuberculous etiology. The clinical features in these two cases are summarized below.

Case 22. W.C., a 29-year-old white male, was admitted to the hospital on May 5, 1949, with a history of migratory polyarthritis and fever for 3 weeks, and a tender,

nodular eruption of the legs of 5 days' duration. He was seen by his physician a week after the onset of his illness and given a course of penicillin with no relief of symptoms. Later he received moderate doses of acetylsalicylic acid which appeared to relieve the arthritis and fever. His physician had noted inflammation of the pharynx, but the patient denied having had a sore throat.

Physical examination revealed typical lesions of erythema nodosum over both legs, but no other abnormalities. The eyes were normal, the parotids were not enlarged, and there were no significantly enlarged peripheral lymph nodes. The liver and spleen were not palpable, and there were no abnormal cardiac findings. The joints appeared to be normal.

X-ray examination of the chest revealed marked bilateral enlargement of the hilar and mediastinal nodes without pulmonary infiltration or cardiac enlargement. The tuberculin test was negative to the first and second strengths of PPD. The blood count was normal, but the sedimentation rate was increased to 36 mm. in one hour. X-ray examination of the hands failed to reveal any abnormalities. Total serum proteins were 6.3, serum albumin 2.8, and serum globulin 3.5 gm. per cent. Throat culture revealed no pathogens, and the antistreptolysin titer was within normal limits.

Needle biopsy of the liver revealed a few moderate-sized granulomata which resembled sarcoids (Fig. 3A). No acid-fast bacilli were demonstrated. Biopsy of one of the skin lesions revealed fat necrosis, lymphocytic and macrophagic infiltration, and surrounding fibroblastic proliferation. No epithelioid or giant cells were seen.

The patient ran a high remittent fever for two and one-half weeks which failed to respond to salicylate therapy. It then fell by lysis and was normal at the end of 3 weeks. During the height of the fever there was an increase in the intensity of the first sound at the apex of the heart, and a soft systolic murmur appeared at the aortic area. Concurrently the P=R interval increased to 0.22 seconds, and there was vague arthralgia without objective signs of arthritis, suggesting the possibility of rheumatic fever. The erythema nodosum lesions began to subside a few days after admission and were completely gone at the end of 3 weeks. The patient was discharged from the hospital on July 26, 1949.

He was seen again eleven months later, on June 1, 1950, at which time he felt well and exhibited no abnormalities on physical examination, except for a small palpable axillary lymph node. X-ray examination of the chest at this time showed a slight decrease in the size of the hilar adenopathy previously described. The electrocardiogram still showed a P=R interval of 0.22 seconds.

Comment. This case illustrates the difficulty of interpreting the significance of hepatic granulomata in erythema nodosum. The histology of the lesions was compatible with either sarcoidosis or tuberculosis. Tuberculosis appeared to be adequately excluded, but the negative tuberculin test, the long duration of the hilar adenopathy without symptoms and the reversal of the albumin-globulin ratio were compatible with sarcoidosis. However, many of the clinical features suggested a streptococcal infection complicated by rheumatic fever, conditions in which sarcoid-like lesions have not been described. The possibility that the erythema nodosum was an unrelated complication of a hitherto unrecognized sarcoidosis could not be excluded, but seemed unlikely in view of the frequent association of erythema nodosum and hilar adenopathy. It is of interest that the histological appearance of the erythema nodosum skin lesions did not resemble those of sarcoidosis, a feature noted by Löfgren,[88] even in cases presumed to be due to sarcoidosis.

Case 23. M.B., a 32-year-old Negress, was admitted to the hospital on June 1, 1950, complaining of painful nodules on her legs of two weeks' duration. For the preceding four months she had been troubled with constant, bilateral, flank pain aggravated by movements of the spine. About two weeks before the onset of her rash she began to complain of increasing fatigability, anorexia, progressive weight loss, fever, night sweats, dry cough, dyspnea, and pleural pain.

Physical examination revealed many typical lesions of erythema nodosum over the posterior and lateral aspects of both legs, signs of a large pleural effusion on the right, and signs of Pott's disease of the thoracic spine. The liver was slightly enlarged, soft and non-tender, but there was no splenomegaly or lymph-adenopathy.

X-ray examination confirmed the presence of a large pleural effusion on the right and revealed extensive destruction of the 11th and 12th dorsal vertebrae compatible with the diagnosis of tuberculosis. The tuberculin test was strongly positive. Aspirated pleural fluid revealed a specific gravity of 1.024 and a cell count of 3,500 per c.m.m., of which 95 per cent were lymphocytes. Guinea-pig inoculation of the sediment failed to reveal evidence of tuberculosis. Gastric washings were likewise negative for acid-fast bacilli. Liver function tests revealed the following: one-minute serum bilirubin .04, total bilirubin 0.30 mg. per cent; cephalin-cholesterol flocculation 1+, thymol turbidity 6.0 units; serum alkaline phosphatase 6.8 Bodansky units; urine urobilinogen 0.15 Ehrlich units per 100 cc., bile negative; serum proteins 6.8, serum albumin 2.45, serum globulin 3.73 gm. per cent. The sedimentation rate was 48 mm. in one hour.

Liver biopsy revealed many small and large granulomata (Fig. 3B), most of which resembled sarcoids and exhibited a reticulum network. Others were irregular in shape with indistinct margins and small areas of necrosis, suggesting tubercles, although no acid-fast bacilli were demonstrated.

Comment. The nature of the vertebral lesion, the lymphocytic pleural exudate, the strongly positive tuberculin reaction and the close association between the appearance of constitutional symptoms and the rash strongly suggested that the erythema nodosum in this case was due to tuberculosis. Presumably, the hepatic granulomata were also tuberculous and indicated hematogenous dissemination, as suggested by van Beek and Haex.[59] However, their histological features did not differ significantly from those in case 22, in which the erythema nodosum was regarded as non-tuberculous. Of special interest was the occurrence of hepatic granulomata in the absence of hilar adenopathy.

It is evident, therefore, that hepatic granulomata may occur in both tuberculin-negative and tuberculin-positive erythema nodosum, that they may be present in the absence of hilar adenopathy, and that their presence cannot be interpreted as proof of a tuberculous etiology, much less of a recent hematogenous spread.

Brucellosis. There is clinical and pathological evidence that the liver is frequently involved in brucellosis. Hepatomegaly,[68] alterations in hepatic function,[69] and jaundice[65] often accompany the disease, and autopsy studies of the liver usually reveal scattered granulomata, focal necrosis, and periportal cellular infiltration.[70] Cirrhosis is an occasional complication,[71-74] and it is thought by some that brucellosis is the direct cause,[75] or an important contributory factor in its pathogenesis.[69] Recently Spink and his associates[69] have reported on the liver biopsy findings in 10 patients with active brucel-

losis. Granulomata were demonstrated in every instance and were usually associated with a cellular infiltrate, especially in the portal areas. They emphasized that the granulomata were nonspecific and could not be distinguished from those of sarcoidosis and, less commonly, from those of tuberculosis and syphilis. Moreover, they made the important point that the diagnosis of sarcoidosis should not be made on the basis of hepatic lesions unless brucellosis has been excluded. Their attempts to culture brucella organisms from liver tissue failed.

Only 2 cases of brucellosis were available for study during this investigation. In one the disease was inactive and no hepatic lesions were demonstrated. In the other, liver biopsy revealed granulomata histologically and brucella organisms on culture. The clinical features in the latter case are summarized below.

Case 24. M.A., a 37-year-old white male, was admitted to the hospital on February 7, 1950, complaining of fever, drenching night-sweats, cough, and chest pain of four days' duration. He was known to have had an attack of brucellosis, complicated by acute cholecystitis, in 1947. In the interim he had felt well except for occasional flatulence and one brief bout of fever. Although the patient appeared ill, no abnormalities were noted on physical examination. The liver and spleen were not palpable.

Blood cultures were positive for *B. abortus* on 6 occasions. Similar organisms were also cultured from aspirated liver tissue, bone marrow, and urine. Brucella agglutinin tests were persistently negative. The gall bladder was not visualized after a double dose of Diodrast. X-ray examination of the chest revealed no abnormalities. Liver function tests revealed a total serum bilirubin of 0.4 mg. per cent, cephalin-cholesterol flocculation 2+, thymol turbidity 2.0 unit, serum alkaline phosphatase 4.7 Bodansky units, bromsulphthalein retention 11 per cent in 45 minutes, and prothrombin 64 per cent. Total serum proteins were 6.7 gms. per cent.

Liver biopsy before treatment revealed many large and small granulomata. Some resembled the lesions of sarcoidosis very closely (Fig. 4A); others were more irregular, exhibited more necrosis, and were more heavily infiltrated with inflammatory cells than is usual in that disease (Figs. 4B,C). The portal triads were moderately thickened by proliferating connective tissue and moderate round cell infiltration, but the lobular architecture was not distorted. There were also small focal areas of round cell infiltration within the lobular parenchyma. The biopsy was repeated one week and 7 weeks following a two-week course of aureomycin therapy. On both occasions the liver still showed small granulomata.

Beryllium intoxication. Following exposure to beryllium some individuals develop severe pulmonary symptoms due to a granulomatous pneumonitis.[76]

Autopsy studies in fatal cases indicate that the granulomatous process is not confined to the lungs, for miliary granulomata resembling sarcoids have been described in a number of tissues including the liver.[29-77] The resemblance to sarcoidosis is not limited to the histological picture, but extends to many of the clinical features, and especially to the roentgenological appearance of the lungs.[78] Scadding,[48] who believes that sarcoidosis is a specific tissue response to a variety of unrelated etiological agents, has suggested that beryllium sarcoidosis is only one of the several types that may occur, and has indicated the necessity for excluding beryllium intoxication in in-

vestigating the nature of any case of sarcoidosis. Until recently it has been assumed that a careful occupational history sufficed to exclude exposure, but the recent report,[77] supported by postmortem histological and chemical evidence, that beryllium intoxication may occur in individuals living near plants employing beryllium compounds, has greatly complicated the diagnostic problem. The possibility of such exposure must, therefore, be borne in mind in interpreting sarcoid-like granulomata found in liver biopsy material.

Only one case of presumed beryllium intoxication was available for investigation.* There was a definite occupational history of exposure to beryllium compounds for two years, following which the patient developed progressive dyspnea, orthopnea, cyanosis, and cough. When seen five years later he was completely incapacitated and presented the signs of a decompensated cor pulmonale with polycythemia, hepato-splenomegaly, clubbing of the fingers, and edema. Liver function tests were normal except for 8 per cent bromsulphthalein retention at 45 minutes, and a 2+ cephalin-cholesterol flocculation. X-ray examination revealed a fine, diffuse, nodular infiltration of both lungs compatible with beryllium pneumonitis, and cor pulmonale. Biopsy of the liver failed to disclose the presence of granulomata.

The diagnosis of beryllium intoxication appears to have been reasonably well established in this case. However, the x-ray findings in the chest and the cardio-respiratory symptoms were also considered compatible with an advanced sarcoidosis. It was only the occupational history which differentiated the two diseases. Even if the liver biopsy had been positive for granulomata it would not have aided in the differential diagnosis, since the lesions in the two diseases are indistinguishable.

Syphilis. Granulomata are said to occur in the liver during early acquired syphilis,[80] and according to Spink and his associates[68] may occasionally resemble those of brucellosis and sarcoidosis. No cases of primary or secondary syphilis were available for study during this investigation.

Leprosy. Miliary granulomata are frequently found in the liver in leprosy,[37] and may be very difficult to differentiate from sarcoids. Indeed, the clinical and histological features of sarcoidosis may be mimicked so closely by leprosy that the lepra bacillus or a related organism has been suggested as the etiological agent in sarcoidosis.[79] Unfortunately, no subjects were available for investigation.

Virus infections. A number of investigators have suggested a virus etiology in sarcoidosis,[7, 80] and recently Löfgren and Lundbeck[41] have reported the isolation of a specific virus from gastric washings and biopsy material in six cases of sarcoidosis. Moreover, miliary granulomata may occur in other virus infections, and may occasionally give rise to difficulties interpreting liver biopsy findings.

* Since this paper was written granulomata have been demonstrated in biopsy tissue obtained from the liver and the skin of a patient with beryllium pneumonitis.

The liver is frequently affected in infectious mononucleosis, a presumed virus infection. Clinically, hepatomegaly and altered liver function occur with great frequency and jaundice is a not uncommon complication. Histologically, the liver usually shows marked hyperplasia of the reticulo-endothelium, and the sinusoids are packed with actively proliferating abnormal lymphocytes, simulating the picture of leukemia.[18, 81] Focal areas of parenchymal necrosis may occur and are usually infiltrated with large mononuclear cells.[82] These may resemble epithelioid granulomata, and, indeed, in the bone marrow granulomata indistinguishable from those of brucellosis, sarcoidosis, and tuberculosis have been described.[58] It is quite possible that similar granulomata may occur in the liver and make the differential diagnosis from sarcoidosis difficult.

Liver biopsy was performed in two non-jaundiced cases of infectious mononucleosis. Both showed the usual changes described, and in addition in one (case 25) there were small sinusoidal collections of large mononuclear cells resembling small atypical sarcoids (Fig. 5A).

Similar small granulomata were demonstrated in the liver during the acute phase of an influenza B infection in case 26 (Fig. 5B), and during the course of an acute febrile illness associated with a rash, thought to be viral in nature (case 27, Fig. 5D).

Mycotic infections. A number of disseminated mycotic infections give rise to visceral and pulmonary granulomata. Of these, histoplasmosis most closely resembles sarcoidosis, both histologically and clinically.[83–84] Granulomata have been described in the liver, amongst other tissues, so that the possibility of histoplasmosis must be considered when sarcoid-like lesions are demonstrated by liver biopsy. Typical *Histoplasma capsulatum* are often found within reticulo-endothelial cells in the liver, but they may be difficult to demonstrate without special stains[84].

One case of histoplasmosis with extensive pulmonary involvement and a strongly positive histoplasmin skin test was investigated. The clinical features suggested sarcoidosis as an alternative diagnosis. Liver biopsy failed to disclose any lesions.

A case of disseminated actinomycosis was also investigated (case 28). The disease was of many years' duration and had involved the subcutaneous tissues, lymph nodes, lungs, and bone before *Actinomyces bovis* was recovered. A liver biopsy revealed several, small, mid-zonal granulomata composed of epithelioid cells (Fig. 5C). No giant cells, actinomyces, or caseation were evident.

Hepatic granulomata have also been described in coccidioidomycosis,[85] and blastomycosis,[85, 86] amongst mycotic infections, and in granuloma inguinale[80] and tularemia.[81] However, neither the clinical nor the histological features in these diseases are sufficiently like sarcoidosis to cause difficulties in diagnosis. Moreover, in all but tularemia the etiological agent can be demonstrated in histological sections of infected tissues.

"Primary" liver disease with sarcoid-like hepatic lesions

In the course of investigating a large number of patients with overt liver disease by needle biopsy, sarcoid-like hepatic granulomata were found in 4 subjects, in whom the diagnosis of sarcoidosis had not been suspected, and in whom no other clinical or histological evidence of the disease could be found. These findings were difficult to interpret and several possibilities were considered.

In occasional cases of sarcoidosis the hepatic lesions may predominate and simulate the picture of primary liver disease, as in case 5. However, typical sarcoids can usually be demonstrated in other tissues in such cases. Although there are clinical reports of sarcoidosis affecting a single organ, they have seldom been confirmed by postmortem examination. It is obvious, however, that many lesions may escape detection by the techniques available to the clinician. It was possible, therefore, that some of the group under consideration fell into this category. Under such conditions, the hepatic granulomata would be indicative of a sarcoidosis either coincidental to, or provocative of the underlying liver disease. There is evidence in the literature to support both possibilities. Scadding and Sherlock[20] reported the occurrence of granulomata in a liver biopsy specimen obtained from a subject with carbon tetrachloride poisoning. The lesions were interpreted as evidence of sarcoidosis, although no other signs of the disease were found. Moreover, sarcoidosis has been reported at autopsy as an incidental finding unrelated to the cause of death.[11, 13, 28] On the other hand, reports of the occurrence of jaundice[7, 22, 28] and cirrhosis[8, 15] during the course of frank sarcoidosis, suggested that the underlying liver disease might be secondary to the granulomata in some of the present group. Finally, in view of the difficulty in distinguishing between the various types of hepatic granulomata, the possibility was considered that the lesions were not due to sarcoidosis, but represented non-specific reactions to the various etiological factors responsible for the underlying liver disease.

The following 4 case reports illustrate the possible diverse interpretations of hepatic granulomata found in subjects with overt liver disease and no other evidence of sarcoidosis.

Case 29. F.F., a 30-year-old white male, was admitted to the hospital on June 22, 1949, because of jaundice of two days' duration. Six days before admission his illness had begun with anorexia, nausea, and mild arthritic symptoms. He had enjoyed good health prior to the onset of his acute illness.

Physical examination revealed moderate jaundice and a slightly enlarged tender liver. There were no skin nor mucous membrane lesions, lymphadenopathy, splenomegaly, ocular lesions, or parotitis.

The leukocyte count on 6/23/49 was 8,500 with 35 per cent neutrophils, 64 per cent lymphocytes, of which several were regarded as atypical, and 1 per cent monocytes. Five days later the count was 6,200 with 67 per cent neutrophils, 29 per cent lymphocytes, and 4 per cent eosinophils. Heterophile agglutinins were positive 1:28 on 7/1/49, 1:28 on 7/30/49, and 1:14 on 8/26/49. Brucella agglutinins were negative. The tuberculin reaction was negative to the first strength and positive to the second strength of

PPD. The total serum proteins were 6.75, the albumin fraction 3.24, and the globulin fraction 3.51 gm. per cent on 6/25/49. These were restudied at frequent intervals and continued to show a slight elevation of globulin. On 8/25/49, at which time the jaundice had completely subsided, the total serum proteins were 6.99, the albumin fraction 3.37, and the globulin fraction 3.62 gm. per cent. X-ray examination of the chest, hands, and feet revealed no abnormalities. Liver function tests were carried out at frequent intervals. Only a few representative studies are given below:

	6-24	*6-30*	*7-14*	*7-28*	*8-11*	*8-25*	*9-21*
One-minute serum bilirubin, mg.%	2.78	1.18	0.31	0.14	0.13	0.07	0.05
Total serum bilirubin, mg.%	5.42	2.71	0.69	0.55	0.55	0.35	0.55
Cephalin-cholesterol flocculation	3+	3+	3+	2+	2+	2+	2+
Thymol turbidity, units	19.3	26.5	17.5	14.5	17.5	25.5	17.0
BSP retention, % at 45 minutes	..	..	..	19.0	22.0	14.5	6.5
Urine bile	4+	3+	0	0	0	0	0
Urine urobilinogen, Ehrlich units/2 hrs.	1.9	39.2	3.0	3.0	1.8	1.6	2.9

A liver biopsy performed on 7/27/49, the 42d day of disease, revealed a large number of granulomata compatible with the diagnosis of sarcoidosis, and minor changes in the portal zones and parenchyma indicative of a healing hepatitis (Fig. 6A).

The clinical diagnosis was infectious hepatitis, and the patient was treated accordingly. The course was afebrile and the patient made a rapid and uneventful clinical recovery, all clinical signs of hepatitis having subsided in two weeks. However, minor abnormalities in liver function, as indicated above, persisted until the date of his discharge from the hospital, 99 days after the onset of his illness.

The patient was readmitted to the hospital two months later for re-evaluation. He felt perfectly well at the time, and physical examination revealed no abnormalities. Liver function tests were carried out and all were within normal limits. The x-ray examination of the chest was still negative. The liver biopsy was repeated and once again sarcoid-like granulomata were found, but there were no signs of hepatitis.

Comment. The history and clinical course in this case were typical of infectious hepatitis. The initial blood count and the positive heterophile agglutinin test, however, suggested the possibility of infectious mononucleosis, a disease which might account for both the acute hepatitis and the granulomata. However, the absence of lymphadenopathy, splenomegaly, and sore throat, and the low titer of heterophile agglutinins were very much against the diagnosis. Moreover, it appeared unlikely that granulomata due to infectious mononucleosis would be found two months after recovery. As for the blood picture, it is well known that the differential white count in infectious hepatitis may resemble that in infectious mononucleosis. On the other hand, hepatic granulomata have not been described in infectious hepatitis, either in the acute phase or during convalescence[87, 88]; nor could the attack of jaundice be attributed to sarcoidosis in view of the prompt recovery despite the persistence of granulomata in the liver. It seemed highly probable, therefore, that this was a case of infectious hepatitis superimposed on an unrelated sarcoidosis. No other evidence of sarcoidosis was found, but deep-seated lesions in inaccessible lymph nodes or viscera could not be excluded.

Case 30. W.S., a 49-year-old white male, was admitted to the hospital on February 2, 1950, because of abdominal pain. For 2 years he had had typical symptoms of chronic cholecystitis and cholelithiasis, and for 4 months symptoms of diabetes mellitus. He had also had a chronic cough for many years, and had spent three months in a tuberculosis sanatorium, where no evidence of active tuberculosis could be found. There was a history of moderate alcohol intake, but the diet had been adequate until anorexia developed six months before admission. The patient's mother had died of diabetes mellitus at the age of 56.

Physical examination revealed moderate obesity, a moderately enlarged, firm liver with tenderness over the gall bladder area, and slight splenomegaly. There were no skin lesions, pulmonary signs, lymphadenopathy, ocular abnormalities, or parotitis to suggest sarcoidosis, and no collateral clinical evidence of cirrhosis, such as icterus, spider nevi, gynecomastia, enlarged abdominal veins, ascites, or edema.

Urine analysis revealed mild albuminuria and marked glycosuria, but no other abnormalities. The fasting blood sugar was 293 and the serum cholesterol 165 mg. per cent. X-ray examination of the chest and hands revealed no abnormalities. Five sputum concentrates and three gastric washings were negative for tubercle bacilli on stain, culture, and guinea-pig inoculation. The tuberculin test was negative to the first strength, but was not repeated with the second strength of PPD. The total serum proteins were 6.3, the albumin fraction 3.7, and the globulin fraction 2.6 gms. per cent. Serum calcium was 9.8, serum inorganic phosphorus 3.0 mg. per cent. Liver function tests performed shortly after admission revealed: total serum bilirubin 0.5 mg. per cent, cephalin-cholesterol flocculation 1+, thymol turbidity 2.0 units, serum alkaline phosphatase 3.4 Bodansky units, and bromsulphthalein retention 26.5 per cent at 45 minutes. Six weeks later these tests were repeated with almost identical results, except for a decrease in bromsulphthalein retention to 16 per cent. Bleeding, clotting, and prothrombin times were normal. A cholecystogram disclosed the presence of cholelithiasis. A roentgenogram of the esophagus failed to demonstrate varices.

Liver biopsy revealed: (a) derangement of the lobular architecture by marked interlobular fibrosis and fatty metamorphosis compatible with Laennec's cirrhosis, (b) vacuolization of the parenchymal-cell nuclei, such as is often seen in diabetes mellitus, and (c) a single large granuloma composed of epithelioid cells and central giant-cells, surrounded by a rim of fibroblasts and lymphocytes (Fig. 6B). There was no evidence of necrosis, and no acid-fast bacilli were demonstrated on stained section. One giant-cell contained a poorly defined asteroid body. Liver biopsy was repeated in 6 weeks with essentially the same findings.

Comment. There seems to be little doubt that the symptoms in this case were due to cholelithiasis and diabetes mellitus. The type of cirrhosis found at biopsy could have been related to either the diabetes or the alcoholism, or to both. The diabetes appeared to be the more important factor, since the alcohol intake had been neither very large nor accompanied by an inadequate diet. The family history of diabetes mellitus militated against the possibility that the diabetes was secondary to hepatic or biliary disease. The histological features of the hepatic granulomata and the absence of other known causes for such lesions suggested sarcoidosis, although no other clinical or histological evidence of the disease could be demonstrated. It was unlikely that the cirrhotic picture was secondary to granulomatous infiltration, in view of the paucity of the lesions and the presence of two other more common etiological factors. It appeared probable, therefore, that

the sarcoidosis was an incidental finding in a patient with diabetes and cirrhosis. Whether the sarcoidosis was confined to the liver is not certain, but it appears unlikely in the light of past autopsy experience.

*Case 31**. L.W., a 38-year-old white woman, first consulted her physician in February, 1949, because of nervousness, migraine headaches, and abdominal distension of about 8 months' duration. Physical examination revealed marked hepatomegaly and splenomegaly. No other abnormalities were noted.

The patient was admitted to a hospital where the following laboratory data were obtained: erythrocyte count 3.73 million, hemoglobin 10.65 gms., leukocyte count 5,000, differential count normal; sedimentation rate 43 mm. in one hour; urine analysis normal except for a trace of albumin. Total serum proteins were 6.25 gms. per cent with 3.25 gms. of albumin and 3.0 gms. of globulin. The serum cholesterol was 297 mg. per cent. Liver function tests revealed a total serum bilirubin of 0.75 mg. per cent, cephalin-cholesterol flocculation 3+, bromsulphthalein retention 20 per cent at one-half hour, and prothrombin 75 per cent of normal. X-ray examination of the chest, gastrointestinal tract, gall bladder, and spine revealed no abnormalities. Brucella agglutination tests were negative.

A biopsy of the liver was obtained through a small abdominal incision. Stained sections revealed an extensive granulomatous infiltration tending to disrupt the normal lobular architecture (Fig. 6C). Close to the capsule the granulomata were circumscribed and confined to the portal zones, which exhibited increased fibrosis and lymphocytic and eosinophilic infiltration. In the deeper layers, however, the granulomata were less well defined and occurred in both portal and mid-zonal areas. The lesions were composed of loosely arranged epithelioid cells, large numbers of giant cells, both of the foreign-body and Langhans' type, and were infiltrated and surrounded by many lymphocytes and a few eosinophiles. No areas of caseation necrosis were seen, but several of the lesions contained small foci of fibrinoid degeneration.

Following her discharge from the hospital early in 1949, the patient was treated symptomatically and appeared to improve. In July, 1950, approximately two years after the onset of her illness, she developed severe hematemesis and required a large number of blood transfusions before her symptoms abated. The hemorrhage was thought to be due to a ruptured esophageal varix.

Comment. This case illustrates that granulomatous infiltration of the liver may be of sufficient severity to simulate the picture of cirrhosis. The histological features were compatible with sarcoidosis. While no other features of the disease were demonstrated, it seemed probable that the spleen was also involved. The poorly circumscribed character of the lesions and the extensive lymphocytic reaction around them suggested the possibility of brucellosis and tuberculosis. However, the afebrile clinical course and the negative agglutination tests were against the former, and the negative chest x-ray, the absence of pulmonary symptoms, fever, or adenopathy appeared to exclude the latter. The increased periportal fibrosis and the disruption of the lobular architecture by the granulomatous process clearly indicated how granulomatosis of the liver could lead to cirrhosis during the phase of healing. The sudden appearance of massive hematemesis

* The authors are indebted to Dr. O. L. Kirklin, Indianapolis, Indiana, for the clinical data and microscopic sections in this case.

compatible with rupture of an esophageal varix suggested that such progression had occurred in this case.

Case 32. N.P., a 54-year-old white male, was admitted to the hospital on November 21, 1949, complaining of painless jaundice and weakness of two weeks' duration. These symptoms were accompanied by dark urine, clay-colored stools, mild gaseous distention, and a 16-lb. weight loss. There were no chills, fever, biliary colic, anorexia, nausea, vomiting, or indigestion.

A history was obtained of "liver trouble" in 1926, but the patient could recall no details of this illness, and denied having had gastrointestinal symptoms prior to the onset of his present illness. The patient had consumed a minimum of one quart of beer daily for some time, but the diet had been good. For several months the patient had eaten 4 to 5 unwashed apples daily, which had previously been sprayed with a lead-arsenic mixture.

Physical examination revealed mild hypertension, obesity, moderate icterus of the skin and sclerae, hepatomegaly with a soft non-tender liver edge palpable a hand's-breadth below the costal margin, slight splenomegaly, and small palpable lymph nodes in the right axilla. There were no skin or ocular lesions, parotitis, or pulmonary findings to suggest sarcoidosis, and no collateral clinical signs of cirrhosis.

The blood count and smear were normal. The urine contained bile, 1+ albumin, a trace of sugar, and a few leukocytes. Subsequent analyses continued to show mild albuminuria and a few leukocytes, but no other abnormalities. A fasting blood sugar was 135 mg. per cent shortly after admission, but was never repeated. The non-protein nitrogen was 40 mg. per cent. The Kahn test was negative. Gastric analysis was normal. The feces were brown and contained occult blood. Liver function tests were carried out periodically and are summarized as follows:

	11-20	*12-5*	*12-9*	*12-29*	*1-2*	*1-26*
One-minute serum bilirubin, mg.%	5.55	1.95	1.35	0.85	...	0.20
Total serum bilirubin, mg.%	7.90	2.60	2.40	1.10	0.50	0.40
Cephalin-cholesterol flocculation	3+	...	3+	1+	1+	0
Thymol turbidity, units	10.1	...	3.75	1.0	1.25	2.25
Serum alkaline phosphatase, units	6.5	...	4.0	2.9	6.4	3.6
Bromsulphthalein retention, 45 min., %	...	14.0	14.0	11.0	1.0	2.5
Prothrombin, per cent	48	68	52	74	68	58

The total serum proteins were 7.0 gm. per cent. Agglutination tests for brucellosis were carried out on two occasions with negative results. A portion of the second liver biopsy specimen was cultured, but no brucella organisms were recovered. The tuberculin test was negative to the first strength, but was strongly positive to the second strength of PPD.

X-ray examination of the chest revealed cardiac enlargement, but no hilar adenopathy or pulmonary infiltration. Roentgenograms of the esophagus, gastrointestinal tract, and hands were normal. A cholecystogram showed poor filling of the gall bladder and a rounded area of calcification in the liver thought to be a calcified hemangioma.

Liver biopsy performed on November 30, 1949, revealed normal liver tissue except for: (a) a few small granulomata composed of epithelioid-cell clusters, (b) a mid-zonal area of focal necrosis infiltrated with lymphocytes and endothelial cells, (c) scattered hyaline necrosis of individual parenchymal cells, (d) a few dilated canaliculi filled with plugs of bile, and (e) moderate portal fibrosis. The necroses were interpreted as indicative of a focal hepatitis, possibly of toxic origin, and it was recommended that the biopsy be repeated to exclude sarcoidosis, in view of the presence of atypical granulomata. Accordingly a second biopsy was performed on January 24,

1950. This specimen revealed normal liver tissue with a number of circumscribed granulomata, chiefly in the portal areas, composed of epithelioid cells and a few giant cells. The granulomata were interpreted as indicating "probable sarcoidosis" (Fig. 6D).

The patient's course was afebrile, and on bed-rest and a normal diet the jaundice faded rapidly and the liver receded to the coastal margin. His appetite was excellent throughout his hospitalization and he had no complaints. He was discharged from the hospital on February 2, 1950, and was advised to return in two months for further observation.

On March 24, 1950, the patient was brought to the hospital in an ambulance and was dead on arrival. According to his family he had been well until two weeks before his death, at which time he began to complain of constant right upper quadrant pain. They attributed this to his having been drinking hard cider pressed from the same unwashed apples previously described. Three days before his death he disappeared from his home and was found wandering in the woods, after having fallen into a pond, and having been badly exposed. He was returned to his home feeling very weak. On the morning of his death he was found to be in a state of collapse at his bedside. Shortly thereafter he had a massive hematemesis and passed several tarry stools. An ambulance was called and he expired en route to the hospital.

The following is a brief summary of the findings at postmortem examination. There were multiple superficial ulcerations of the mucosa of the lower esophagus and of the lesser curvature of the stomach, with a large amount of clotted blood in the stomach. These were associated with a chronic esophagitis and chronic gastritis, but no esophageal varices were demonstrated. The liver was enlarged and exhibited both gross and microscopic evidence of a fine, diffuse cirrhosis. No circumscribed hepatic granulomata were found in 7 sections, but several showed small collections of epithelioid cells in the portal areas without giant cells. The spleen was slightly enlarged, weighing 375 grams, but contained no granulomata. A group of mediastinal and coeliac lymph nodes were enlarged and were found to contain numerous multinucleated giant cells of the foreign-body type with vacuoles and prominent asteroid bodies, and vacuolated macrophages. There was no associated fibrosis. The heart was considerably enlarged (700 grams), and the lungs were the site of an organizing non-suppurative pneumonitis. There were small collections of serous fluid in the pleural and abdominal cavities. Microscopic examination failed to reveal the presence of granulomata in any of the other viscera or lymph nodes examined.

The tissues and gastric contents were subjected to an exhaustive toxicological study, but no evidence of heavy-metal or alkaloid poisoning was found.

Comment. The history of possible exposure to arsenic, the clinical course, and the initial liver biopsy suggested an acute toxic hepatitis. However, the absence of fatty metamorphosis in the liver and the failure to detect significant amounts of arsenic in the tissues were important points against an acute arsenical hepatitis. While the age of the fibrosis found in the liver could not be estimated with certainty, it seemed more compatible with that of a Laennec's cirrhosis related to chronic alcoholism, than to the residuals of a recent acute hepatitis. In that event the attack of jaundice might be interpreted as indicative of hepatic failure on a cirrhotic basis.

The nature of the hepatic granulomata found on biopsy remains obscure. While they resembled sarcoids, no similar lesions were found at autopsy. The marked infiltration of the mediastinal and coeliac glands with foreign-body giant cells and macrophages did not resemble the lesions of the active phase of sarcoidosis, nor, in the absence of fibrosis, did they resemble those

of the healing phase. This case illustrates the difficulty of interpreting the significance of hepatic granulomata. A presumptive diagnosis of acute toxic hepatitis and coincidental sarcoidosis was made on the basis of the liver biopsy findings. It was not until the postmortem examination was carried out that it became evident that the diagnosis was incorrect. However, even then a thorough investigation of all the tissues failed to elucidate the nature of the granulomatous reaction.

Summary and conclusions

The clinical, functional, and histological status of the liver was investigated in 20 cases of sarcoidosis, and, for purposes of comparison, in a number of other diseases associated with granulomatous lesions.

Submiliary granulomata were demonstrated by needle biopsy of the liver in all 15 histologically-confirmed cases of sarcoidosis, but in none of the 5 with a presumptive diagnosis, and in 10 of the former, histological confirmation of the diagnosis rested on the liver biopsy findings alone. The incidence of hepatic sarcoids demonstrated by needle biopsy did not differ significantly from that observed at autopsy, indicating that the sampling error was small.

Hepatomegaly was present in 11 of the 15 cases exhibiting hepatic granulomata, and was accompanied by splenomegaly in 6. Mild gastrointestinal symptoms and minor alterations in hepatic function occurred in most of the subjects with hepatic sarcoidosis, but the evidence suggested that some of these were related to the systemic effects of the disease, rather than to the presence of specific hepatic lesions. In one subject, however, the granulomatous infiltration was sufficiently extensive to produce moderately severe hepatic dysfunction and symptoms, including jaundice.

Submiliary granulomata were also demonstrated by needle biopsy of the liver in miliary, pulmonary, and osseous tuberculosis, in erythema nodosum, and in brucellosis. These were often associated with slight hepatomegaly and minor alterations in hepatic function, as in sarcoidosis. Smaller, somewhat atypical granulomata were demonstrated in infectious mononucleosis, influenza B, in an unidentified viral infection, and in actinomycosis. No hepatic lesions were found in a subject with beryllium intoxication, or in one with histoplasmosis. The occurrence of miliary hepatic granulomata in early syphilis, leprosy, tularemia, and other mycotic infections was discussed, although no subjects were available for investigation.

There were striking resemblance in morphology between the hepatic lesions of sarcoidosis on the one hand, and those of tuberculosis, erythema nodosum, and brucellosis on the other. Although many of the granulomata in tuberculosis and brucellosis exhibited a greater degree of necrosis and surrounding inflammatory reaction than those in sarcoidosis, most of the lesions could not be differentiated on histological grounds alone. Caseation necrosis and acid-fast bacilli were demonstrated only once each in the

hepatic granulomata of 7 subjects with tuberculosis. Brucella organisms were successfully cultured from the liver biopsy specimen in a subject with active brucellosis.

The hepatic lesions in tuberculin-negative erythema nodosum did not differ from those in tuberculin-positive erythema nodosum, and could not be distinguished from those of sarcoidosis and tuberculosis. The interrelationships between these three diseases were discussed.

Granulomata resembling those of sarcoidosis were discovered on routine liver biopsy in a case of acute hepatitis and in 3 cases of cirrhosis, in whom no other signs of sarcoidosis could be found. The evidence suggested that the lesions were due to a coincidental sarcoidosis in the case of hepatitis and in one of the cirrhotics. In the third case the lesions were also probably due to sarcoidosis, but the cirrhosis appeared to be secondary to the sarcoidal granulomatous infiltration. In the fourth case the nature of the lesions could not be determined, even after postmortem examination, but sarcoidosis appeared to be excluded.

The results of this investigation indicate that needle biopsy of the liver is of great value in confirming the diagnosis of sarcoidosis histologically, especially when readily accessible lesions in the skin and peripheral lymph nodes are not available for study, but that the histological findings must be supplemented by other clinical and laboratory data to exclude granulomatous diseases which resemble sarcoidosis.

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NEWS AND COMMENT

Excerpts from the Minutes of the Board of Permanent Officers

Frederick Noyes Sperry. The Board of Permanent Officers records with regret the death on September 7, 1950 of Frederick Noyes Sperry, M.D., Clinical Professor of Otolaryngology Emeritus and Consulting Surgeon (Otolaryngologist) to the New Haven Hospital.

At his retirement in 1942 at the age of sixty-eight there was spread on the Minutes of the Board an account of his associations with the faculty of the School of Medicine, the New Haven Hospital and the New Haven Dispensary over some forty-eight years. Save for one year of a rotating internship in the hospital immediately subsequent to his graduation from the Yale School of Medicine in 1894, he specialized in the care of patients in the hospital and dispensary. Of the last he was a member of the Board of Directors, Secretary of the Corporation, and Administrator for some years, up to the time the New Haven Hospital assumed this responsibility.

Dr. Sperry's contributions were not, however, limited to these activities. He remained throughout his lifetime a loyal alumnus of both the School and the Hospital under circumstances which were many times sorely trying. For the former he instituted and maintained the Yale Alumni in Medicine allotment for financial aid to needy students and for the latter, through his personal contact with Mr. Fitkin, he took the initial steps in obtaining the monies for the construction of the Fitkin wards and endowment for the support of beds there for children.

In his later years Dr. Sperry was particularly concerned with the organization and development of relief for the "hard of hearing," and not only contributed his professional services but undertook to arouse public interest and support of this endeavor.

While not a prolific contributor to the literature of his specialty, he was recognized at the national level as an able practitioner of his specialty. Evidence of this was his membership in the American College of Surgeons, the American Academy of Ophthalmology and Otolaryngology, and the American Rhino-Otological Society, while at the local level he was an active member of the city, county, and state societies.

SAMUEL C. HARVEY

Lewis Thorne. The medical world and our own medical community were deeply shocked and grieved by the death of Dr. Lewis Thorne. On October 8, 1950 Dr. Thorne was killed by the bullets of a former paranoid patient. His wife, Helen, was seriously injured.

Lewis Thorne graduated from Yale College and from our Medical School (1936). From 1939 to 1950 he worked in the Division of Student Mental Hygiene of the Department of University Health. From 1941 to 1946 he was a medical officer in the Army of the United States. He served in the European theatre as a Lieutenant-Colonel and was distinguished with the Bronze Star for outstanding courage before the enemy.

Dr. Thorne was a kind, gentle man, one of the rare people who had only

friends. While at Yale he helped countless students with their psychological problems. As an Assistant Clinical Professor he taught medical students in the Department of Psychiatry.

. . . Modern psychiatry has become permissive and humane. Patients are no longer kept in chains and dungeons but are given considerable freedom and tolerant treatment. It is inevitable that at times in an atmosphere of permissiveness, tolerance, and lack of suspicion, dangerous patients hiding their anti-social impulses will do harm. Dr. Thorne's death was the toll for such a spirit of permissiveness. His death has stimulated new research about anti-social patients in mental hospitals and in the community. We will remember our friend and colleague Lewis Thorne.

FREDRICK C. REDLICH

HARLAN B. PERRINS. The Board of Permanent Officers of the Yale University School of Medicine records its deep regret in the death of Harlan B. Perrins, Associate Clinical Professor of Obstetrics and Gynecology. Dr. Perrins received his bachelor's degree at Yale in 1916 and his doctorate in medicine in 1918. He served as lieutenant in the United States Navy until 1919 and then entered residency training in obstetrics and gynecology at the Brooklyn Methodist Episcopal Hospital and the New York Lying-in Hospital. He began private practice in his specialty in New Haven in 1921.

Dr. Perrins was appointed Associate Clinical Professor at the School of Medicine in 1936, a position which he filled with high satisfaction and fine devotion to the best interests of the school. As Chief of the Obstetrical and Gynecological service at the Grace Unit of the Grace-New Haven Community Hospital since 1935 he had deep interest in developing a residency training program which has enjoyed a wide reputation for its excellence. In recent years he was active in planning and organization of the Grace-New Haven Community Hospital now under construction. Here he visualized an integration of a post-doctorate training program with the University Service which would be of great mutual interest. During the past year the beginning of such combined effort was put into practice under his cooperation and guidance. He is remembered by his colleagues at the School of Medicine as a distinguished alumnus, a skillful physician, and a cherished friend.

HERBERT THOMS

ALUMNI EDITION OF THE JOURNAL

Beginning with the February, 1951, number of the Journal, a special alumni edition will be published as well as the regular edition. This is the result of an arrangement between the Journal and the Association of Yale Alumni in Medicine whereby the Association will include a subscription to the Journal in its annual dues of six dollars.

The alumni edition will consist of the regular edition plus approximately six unnumbered pages of alumni notes and news about the School. The new edition will be sent only to members of the Association who have paid their dues; all other subscribers will continue to receive the regular edition.

EVENTS

TOXICOLOGY SEMINAR

October 26, 1950

BERYLLIUM—ITS RELATION TO THE PROBLEMS OF NORMAL AND MALIGNANT GROWTH. By Mahlon B. Hoagland, M.D., Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, Massachusetts General Hospital, Boston, Massachusetts.

The property peculiar to insoluble salts of beryllium of inducing osteogenic sarcomas in rabbits was utilized to study some factors involved in neoplastic growth. Tumor tissue exhibited a higher alkaline phosphatase activity than normal tissue. Serum alkaline phosphatase activity paralleled the extent of metastases rather than the size of the primary tumor.

Although the beryllium-induced sarcomas in rabbits were exactly like those arising spontaneously in man, there have been no osteogenic sarcomas reported in humans exposed to the metal. Toxicity in man is manifest by dermatologic lesions and granulomatous lung lesions resembling Boeck's sarcoid. Laboratory animals, on the other hand, did not demonstrate granulomas when treated with the substance, and neither guinea pigs nor rats were susceptible to the sarcomagenic action of beryllium. Transplants from the tumor-ridden rabbit to normal rabbits or guinea pigs failed to grow. Ingestion of beryllium by rats gave rise to a syndrome which had a striking similarity to that of avitaminosis D. The resultant "rickets" was frequently refractory to vitamin D and calcium therapy.

An *in vitro* investigation revealed that beryllium in concentrations as low as 10^{-7} M inhibited alkaline phosphatase activity of bone as contrasted with magnesium which activated this enzyme system. The apparent competition of these two ions was further demonstrated in plants, beryllium suppressing plant growth presumably by replacing magnesium in chlorophyll. In the presence of a deficiency of magnesium, however, beryllium stimulated plant growth.

J. P. GREEN

NEUROLOGICAL STUDY UNIT

October 31, 1950

TESTS OF BRAIN FUNCTIONS. By Ward C. Halstead, Professor of Experimental Psychology, Departments of Medicine and Psychology, University of Chicago.

The problems of intelligence testing are both conceptual and methodological—results in the present day furnishing more information about the subject's "intellectual activity" than his "intellectual level" as originally distinguished by Binet. Elements of the former are said to be eliminated in favor of the latter by tests similar to the Flicker Fusion test. Data comparing controls, frontal lobotomy patients, and non-frontal lobotomy patients were presented indicating their reactions to Flicker Fusion and Category tests. Further experimental evidence shows that the Flicker Fusion test varies as well with such conditions as time of day or year and the physiological state of the individual. Thus the phenomenon used correlates with both biological activity and behavior to such an extent that some claim to have predicted the onset of depression in a patient by means of critical fusion

frequency. An attempt has been made, then, to circumvent some of the well-known problems of intelligence testing and to obtain quantitative results.

O. L. K.

WOODWARD LECTURE

November 6, 1950

MIND AND MATTER. By Bertrand Russell, Fellow of Trinity College, Cambridge, England.

In this lecture, Russell offered a brief exposition of his views concerning the nature of mind and matter, concepts which, in these days of cybernetics and atomic physics, assume the greatest importance. The discussion was confined entirely to a philosophical approach.

Mind was first contrasted to body in that one's mind appears only to oneself while one's body appears to others. Further consideration of mind led to its definition as a series of occurrences, called thoughts, which are bound together by the laws of psychology. The definition of thoughts is implicit in this definition of mind, as being the series of events making up the mind. However, thoughts are to be distinguished from other events, sensation and perception. The nature of sensation and perception and their distinction from one another were explained by Russell as resulting from the presence of two different factors in each of us with which we react to any stimulus from the outside world. There is: (a) a part of our structure which we have in common with all other individuals, and (b) a factor which is the result of past experience. As a result of stimulation, we receive a sensation. This is due to (a) alone. Perception, however, is the total occurrence in us resulting from the stimulus, and this is due to (a) and (b) together.

This naturally led to a distinction between the physical world of physics and the physical world of daily experience. The former consists of events outside our mind. This world is solid but cannot be absolutely verified, since these events can only serve to cause events in us, thus constituting a part of our physical world of daily experience. This latter, while not at all solid, is all that we are sure of. Therefore, we have the apparent paradox that "the physical world of physics is solid but dubitable, while the physical world of daily experience is not solid, but is indubitable."

From these definitions a final consideration concerning mind was discussed—the difference between life and non-life. The distinction was made that, while both undergo transformations, only life can experience these transformations. Experience is to be thought of as the memory of past events modifying present behavior, while memory is the knowledge of perceptions, i.e., to have a memory of a perception, you must actively note the perception in some way (e.g., verbally) at the time of occurrence.

After thus considering the nature of mind and the many ramifications arising from this, matter was dealt with quite briefly. Matter is something that we know of from its space-time relations, or put into terms analogous to our definition of mind, matter is a series of occurrences bound together by laws of physics. The most modern concept of these occurrences is that they are represented by the sudden changes in an energy distribution.

In summary, then, mind and matter are each a series of events, the former being bound together by the laws of psychology, the latter by the laws of physics, i.e., we know the space-time relations of matter, but not of mind. Mind, furthermore, is connected causally with objects of the world outside

oneself, but all experience is inside one's brain. (Mind and brain, Russell considers, differ only in their grouping of events, somewhat analogously to the difference between an alphabetical and a geographical listing of individuals in a city.)

As a final consideration, Russell presented the possibility of disembodied mind, which would after all, only be the antithesis of inanimate matter. That is to say, inanimate matter conforms to laws of physics, but not to laws of psychology. Disembodied mind, then, would simply be events that conform to laws of psychology, but not to laws of physics.

R. G.

BIOPHYSICS SEMINAR

November 14, 1950

THE BIOPHYSICS OF VISUAL PURPLE. By J. C. Peskin, Institute for Visual Research, University of Michigan, Ann Arbor, Michigan.

The mechanism of vision may be divided into two parts; first, a photochemical reaction in the retina and, secondly, neuronal transmission and interpretation. While differing widely in anatomic design, the process of vision shows similarities among diverse classes of vertebrates and invertebrates. Among these are the presence of carotinoids, intensity thresholds of response, and spectral sensitivity. The basis of these similarities is the common nature of the photochemical compounds present in all visual systems. These compounds have been examined by two methods—direct chemical extraction and examination, and testing of the responses of the intact visual system.

The photochemical compound about which most is known is visual purple in the frog, found in the outer limbs of the rods of the retinae. Electron microscopic studies on dry, unfixed rods show a structure of beads strung on strands running lengthwise in the outer limb. Chemical analysis reveals visual purple to be compounded of protein plus a carotinoid residue. It is extraordinarily sensitive to heat, acid and alkali, and salts of heavy metals, though resistant to trypsin and putrefaction, and is best extracted with petroleum ether. Molecular weight determinations run from 270,000 to 800,000. No unusual spectrum of amino acids is found. The bleaching reaction is the separation of the protein from the carotinoid retinene, which subsequently transforms to vitamin A. The rate of bleaching by light is unaffected by temperature. The energy release in bleaching is considerably less than the energy of neuronal discharge; intermediate steps are completely unknown. Further, full bleaching is probably not necessary for the required energy transfer.

Regeneration of visual purple *in vitro* is seen to occur after bleaching by blue light, but not following yellow light. Rate of regeneration is reduced by intensifying the prior exposure, lengthening exposure, or lowering the temperature during regeneration. The curve is that of an autocatalytic reaction.

Studies on dark adaptation in man reveal confirmatory data. The rapid recovery following a dim light is autocatalytic in character. Slow adaptation following longer, more intense exposure is best represented by bimolecular kinetics. These conclusions are drawn from the "rod" part of the adaptation curve, that is the portion following in time complete adaptation of the cones. A few completely color-blind persons, presumably with absent or non-

functional cones, have given opportunity for study of the rod curve in its entirety.

Vitamin A in massive doses does not affect the vision of most normal persons, though in a few cases it does raise night vision to supernormal acuity.

W. P. McN.

YALE CHAPTER OF SIGMA XI

November 3, 1950

SEEING VIRUSES AND THE MACRO MOLECULES OF LIVING MATTER. By Ralph W. C. Wyckoff, Laboratory of Physical Biology, National Institute of Health, United States Public Health Service.

In the eleven years the electron miscroscope has been in use, this instrument has proved to be our most valuable tool for investigating macro molecules. It affords a direct check on the more indirect data from ultracentrifugation, plus allowing study of insoluble macro molecules (as for example connective tissue) which are not amenable to centrifugation. It has introduced a new discipline, which may be termed "visual chemistry." The processes of synthesis, degradation, and crystallization may be observed stepwise at a molecular level. Viruses may easily be recognized, and electron microscopy produces the most direct information regarding viral replication and the induction of cellular pathology.

The limiting resolution of existing electron microscopes defines a particle ten to 15 Å in linear dimension. This is a magnification which bears the same ratio to that of the optical microscope as does the latter to naked eyesight. It probably will be decades before we can understand much of the detail we now see in electron micrographs.

Numerous excellent lantern slides of micrographs were demonstrated. Clearly seen are two principal types of macro molecules—spherical and rod-shaped. The latter may be thick and stiff, as tobacco mosaic virus, or filamentous and wavy, as cellulose. Micrographs of three-dimensional protein crystals clearly indicate individual molecules in the array as well as the mode of packing. Some substances form "paracrystals," or two-dimensional arrays.

Thin slices of tissue show lacy framework in solid-liquid gels, as protoplasm, and regularly interwoven bundles in solid-solid gels, as connective tissue. Muscle tissue shows a two-component, three-dimensional array of bundles.

Among pictures of phage adsorption on and subsequent lysis of bacteria was a striking view of formed phage particles in the interior of a bacterium from which a single thin slice was taken. Tobacco mosaic and fowlpox viruses are distinctly seen intracellularly in their respective host tissues.

W. P. McN.

PHARMACOLOGY SEMINAR

November 9, 1950

MICROBIOLOGICAL ASPECTS OF DRUGS AND ANTIBIOTIC RESISTANCE. By Henry P. Treffers, Professor of Microbiology, Yale University.

Some drugs and antibiotics inhibit bacterial growth by interfering with the synthesis, utilization, or assimilation of metabolites. By producing the essential metabolite in greater quantity, microorganisms may adjust to the

presence of the antibiotic in the medium and may even become dependent upon the drug. This dependence may be explained, it was postulated, by an excessive, toxic accumulation of the metabolite in the absence of the inhibiting agent.

Development of bacterial resistance to chemical substances cannot at present be explained by an all-inclusive theory because of the difficulty of extrapolating the evidence gained from one organism to explain the behavior of another. Gram positive organisms adjust to the interference by penicillin of glutamic acid assimilation by changing to gram negative organisms which do not require glutamic acid in the media. But higher levels of penicillin inhibit gram negative organisms, too, indicating a different mode of action. Penicillinase production may, in part, account for resistance, but no general relationship between resistance and production of the enzyme has been demonstrated by all bacteria. Resistance of organisms to one type of penicillin differs only slightly from resistance to other types of penicillin.

Inhibition of growth of *E. coli* by chloromycetin is accompanied by an elevated desoxyribosenucleic acid—ribosenucleic acid ratio, and a loss of these acids from the cell, alterations not exhibited by resistant organisms. The ratio DNA/RNA is not influenced by penicillin.

In organisms sensitive to streptomycin, the coupling of pyruvate and oxaloacetate is inhibited by streptomycin: organisms resistant to this antibiotic do not appear to carry out this reaction. As contrasted with penicillin, which engenders resistance in graded, discrete steps, resistance to streptomycin can be induced in one step.

Quantitative measurements of the degree of resistance are complicated by the variations found in a given species. Susceptibility to chemotherapeutic agents varies among individual organisms in a colony, and the degree of variability differs with each agent. Normal distribution rather than homogeneity prevails among organisms of the same species. One in a million organisms is resistant to streptomycin; if this one organism be cultured, one thousand of the million resultant daughter cells are resistant.

Streptomycyl amines were investigated for activity upon the tubercle bacillus. The N-diamyl and N-phenylbutyl derivatives of streptomycin have less activity than streptomycin, and organisms resistant to or dependent upon streptomycin grow in their presence. The N-decyl and N-cyclohexylbutyl derivatives, on the other hand, do not support the growth of dependent strains, and, further, they retard the growth of organisms resistant to streptomycin. The toxicity of the two compounds precludes clinical use, but it is hoped that useful substances may arise from this approach to chemotherapy.

J. P. GREEN

NEUROLOGICAL STUDY UNIT

December 5, 1950

THE NATURE OF THE DEPENDENCE OF SKELETAL MUSCLE UPON INNERVATION. By D. D. Denny-Brown, James Jackson Putnam Professor of Neurology, Harvard Medical School.

Microscopic examination of denervated muscle reveals the gradual formation of a condition suggesting degeneration rather than mere loss of mass. Previously undescribed pyknotic nuclei are seen arranged in columns, the myofibrils having been lost. To examine the ability of denervated muscle cells to regenerate the missing myofibrils, injury by clamping was

inflicted at different time intervals following nerve section. After three weeks of denervation, attempts at regeneration occurred (for example, budding) but were not sustained. The incidence and effectiveness of regeneration declined as the denervation period prior to injury was prolonged. Myofibrils were not produced as has been demonstrated in similarly injured muscles with intact motor nerves. The implication is, therefore, that the presence of the nerve not only prevents fibrillation but also enables the muscle cell to produce fibrils. The nature of this aspect of the nerve-muscle relationship is, of course, unknown.

In discussion, Dr. Denny-Brown reaffirmed his belief that muscle fibres of different types may be innervated by a single dichotomizing nerve fibre and that whole muscle fasciculi may occasionally be innervated by the branching axons of a single ventral horn cell. In poliomyelitis, therefore, one occasionally sees complete atrophy of a muscle fasciculus while surrounding fasciculi remain normal.

O. L. K.

YALE MEDICAL SOCIETY

December 13, 1950

AN EPIDEMIC OF POLIOMYELITIS CHARACTERIZED BY DUAL INFECTIONS WITH COXSACKIE AND POLIOMYELITIS VIRUS. By Joseph L. Melnick.

In a severe epidemic of poliomyelitis occurring in Easton, Pennsylvania during August and September of 1949, 120 cases were reported among a total population of approximately 100,000, including the city and its environs. Of the 45 hospitalized patients studied, 65 per cent of them excreted poliomyelitis virus, 75 per cent "C" virus, and 50 per cent excreted both viruses. That infection by poliomyelitis and "C" viruses occurred simultaneously was shown by a concomitant rise in blood levels of antibodies to both poliomyelitis and "C" viruses, paralleled by a decreased excretion of "C" virus. Data gathered on epidemics where "C" virus accompanies polio infection may indicate that invasion by both organisms predisposes the patient to paralysis rather than to the subclinical or mild symptoms usually encountered in infection with polio virus alone.

V. S. G.

A QUANTIFIED MULTIPLE DESCRIPTION FOR MENTAL HOSPITAL PATIENTS. By J. R. Wittenborn.

The paucity of adequate methods for the description of behavior has prompted this attempt to quantify and standardize descriptions of mental hospital patients' symptoms. The primary purpose of the study was to provide a tool for research in psychiatry and clinical psychology. A number of psychiatrists were asked what they would consider as evidence for each of the classical diagnoses. Only functional disorders were included. As a result, it was possible to deduce a group of fifty-five rating scales each of which included four descriptive phrases, ranging from the most pathological manifestation to the least. Since it is impractical to work with or understand so many scales at one time, two large samples of patients were rated and factor analyses of the interrelations among the scales revealed that the scales tended to cluster into seven major groups. The groups coincided with plausible diagnoses. Thus, the same points of reference, quantitative in

nature, are available for the purpose of describing a patient's symptom manifestations. Cited applications of the method were the author's study of psychosurgical patients and of the Bellevue-Wechsler Test.

O. L. K.

BUTYL EXTRACTABLE IODINE OF SERUM. By Evelyn B. Man, David M. Kydd, and John P. Peters.

In previous reports the serum precipitable iodine levels (SPI) of hyperthyroid patients treated with antithyroid drugs have been found to be inordinately high in relation to other measures of thyroid activity. A measure for circulating thyroxin more specific than SPI was needed. For this purpose a simplification of Taurog and Chaikoff's method has been used to compare with SPI the butanol soluble "thyroxine-like" iodine of duplicate 1 ml. aliquots of serum from hyper-, hypo-, euthyroid, and pregnant human subjects.

Agreement between the SPI and butanol extractable iodine of serum (BEI) is within 2 gamma per cent, with an average difference of not more than 0.6 gamma per cent, in 30 subjects not receiving therapy for hyperthyroidism. This group of subjects included the euthyroid individuals, the hypothyroid patients taking desiccated thyroid, the pregnant women, and the untreated patients with hyperthyroidism. In contrast, in 57 measurements on 35 patients receiving antithyroid drugs for hyperthyroidism, differences between the SPI and BEI were larger, amounting in a few instances to 10 gamma per cent. False high values occurred in patients who had been taking Lugol's solution; in these patients the height of the butanol extractable iodine usually agreed better than the amount of SPI, with the clinical evaluation of hyperthyroidism.

EVELYN B. MAN.

OBSERVATIONS ON THE HYDRODYNAMICS OF BRONCHIAL-PULMONARY VASCULAR CONNECTIONS. By Ralph D. Alley, Donald P. Shedd, and Gustaf E. Lindskog.

Connections between the pulmonary and bronchial arterial trees have been detected in normal perfused lungs and in casts of diseased lungs. Experiments were therefore conducted to determine the functional patency of these connections.

In acute procedures in 11 dogs, the pulmonary artery and vein of the left lower lobe were equipped with ligatures which could be manipulated from the outside after closure of the thoracotomy wound. A pressure-recording cannula was left in the pulmonary artery distal to the ligature. Occlusion of the vein caused rise in pressure of 3 to 18 mm. Hg. Occlusion of the artery alone produced a fall to zero and a disappearance of the pulse. Occlusion of both produced a rise over several minutes to 40 mm. Hg, followed by prompt return to normal after release of the ligatures. The elevated pulmonary tension varied with changes in the systemic pressure induced by bleeding and by epinephrine. In two dogs where the pulmonary artery to this lobe was ligated six weeks prior to the acute experiment, pressure rise on venous occlusion was more rapid and higher, to 80 mm. Hg.

There can thus be no doubt of connections of appreciable magnitude patent in the living normal lung. The pressure rises were too rapid and too high to be accounted for by capillary connections alone. Precapillary or

even arteriolar anastomoses must exist, though not described by anatomists. The two chronic experiments demonstrate the rapidity with which these connections may enlarge in case of insufficient pulmonary artery flow. Considerable influx of blood at systemic pressure in pulmonary disease may contribute to the development of cor pulmonale. It is possible that infarcts following emboli in congested lungs may depend on duplication by nature of this experiment. The local arterial occlusion together with chronic venous congestion may cause an increase in pressure leading to edema and hemorrhage.

W. P. McN.

THE ILLUSTRATIONS FROM THE WORKS OF ANDREAS VESALIUS. With annotations and translations, a discussion of the plates and their background, authorship, and influence, and a biographical sketch of Vesalius by J. B. deC. M. Saunders and Charles D. O'Malley. Cleveland, World Publishing Company, 1950. 248 pp. $10.00.

Andreas Vesalius (1514-1564) was, more than any other man, the founder of modern anatomy. His greatest work, *De humani corporis fabrica,* was published in 1543. It is a superbly illustrated work, and 73 of the 96 plates in this reproduction contain the illustrations from the *Fabrica.* The other plates are from Vesalius' *Epitome* of the *Fabrica* (1543), his *Venesection Letter* (1539), and his *Tabulae Sex* (1538). However, the plates from the great *Fabrica* are the most important in terms of Vesalius' contribution to anatomy.

The 30-page introductory biographical sketch of Vesalius is excellent. It does not contain any new evidence, but there is no better balanced interpretation of the man. However, the authors did not attempt to relate Vesalius' work to the general history of science or to the cultural history of the sixteenth century. For instance, they do not even mention that another great work in science—Copernicus' *De revolutionibus orbium celestium*—was also published in 1543.

Each illustration is accompanied, usually on the opposite page, by a translation of Vesalius' caption and the authors' annotations. These annotations are well done, and it is evident that the authors have consulted the indices of the illustrations in making their annotations. By so doing they have been able to point out that some of the anatomical errors in the plates were corrected in the text and that other apparent errors were deliberate efforts to portray varying anatomical structure as found in man and lower forms. The authors have not translated Vesalius' indices to the plates "since the terminology employed is so archaic as to require, except for the expert in sixteenth-century medicine, interpretation of almost every term. . . ." And they are correct. However, the value of the translated captions and the annotations would have been greatly increased for those not having a good knowledge of human anatomy had the structures mentioned been identified by the index letters on the plates.

Anyone using this work should know that the translated captions are from the second or 1555 edition of the *Fabrica* although this fact is apparently not mentioned by the authors. One must approve the choice of the 1555 captions over those of 1543 since the former present more completely Vesalius' knowledge of anatomy.

The plates in this edition have been reduced by fifteen to twenty per cent but the reduction does not mar the beauty and detail of the originals. Indeed, this edition is most handsome. It is excellently designed and very well printed. Most important of all, the Vesalian illustrations with their captions and commentary are now widely available in a usable form.

FREDERICK G. KILGOUR

MEDICAL PHYSICS, Volume II. Otto Glasser, Editor-in-chief. Chicago, The Year Book Publishers, Inc., 1950. xxvi + 1227 pp. $25.00.

Coming as a supplement to Volume I which appeared in 1944, this reference text will be welcomed by biophysicists and physicians concerned

with problems in medical physics. Even the biologist and medical man who is not directly concerned with strictly physical investigations but nevertheless depends heavily on physical instrumentation in his laboratory will find here much to his use.

This is not a re-edition of the previous volume. New sections have been added and older sections have been expanded where necessary. For subjects in which little of significance has been added, references are made to title headings and page numbers in Volume I. The largest additions have of course been made in topics related to radiation and radioisotopes. Lengthy treatments, theoretical and instrumental, are given to Geiger counters, radioisotopes, roentgenography, radiation therapy, and protection and health physics. There are also notable essays on electron microscopy, optics, and spectroscopy, the physics of circulation and respiration, aviation medicine, blast injury, and decompression sickness. The list of title headings is far too long to cover in review.

The arrangement is alphabetical, by title headings, with no table of contents. Authors are listed under each heading. Long subject and author indices give adequate cross-references. Bibliographies are included with each section and in general are comprehensive. Names appearing in the bibliographies are included in the author index. The text is profusely supplied with line drawings and half-tones.

As the second volume is incomplete by itself, and the outlay for both is considerable ($40.00) most users will be more interested in knowing that Volume II is available on reference shelves than in purchasing it. Both are worth the price, however, to specialists in medical physics and biophysics.

W. P. McN.

THE PATHOGENESIS AND PATHOLOGY OF VIRAL DISEASES. J. G. Kidd, Editor. New York, Columbia University Press, 1950. 235 pp. $5.00.

A very interesting collection of fifteen papers, by as many authors, well illustrates the diversity of interests, approaches, and methods of the workers in this field. Five articles are classical pathological descriptions treating of the exanthemata (T. F. M. Scott *et al.*,) yellow fever (J. E. Ash), lymphocytic choriomeningitis (R. D. Lillie), the viral encephalitides (Abner Wolf), and respiratory tract infections (T. P. Magill).

Characteristics of virus action are discussed in relation to interference (G. Dalldorf) and to activation by chemically defined substances, "co-factors," (T. F. Anderson), while a study of virus growth by electron photomicrographs is contributed by R. W. G. Wyckoff.

Host reactions are emphasized by J. Buddingh, who describes the varying responses to infection of the chick embryo according to age, route of inoculation, and other factors. H. A. Howe reports on poliomyelitis in monkeys, combining descriptive pathology, an account of virus localization and multiplication following selective destruction of portions of the central nervous system, and initial attempts to characterize chemically the changes in pathological areas.

The reviewer found particularly provocative Shope's discussion of epidemics. This author is concerned, not with the conventional treatment of the subject—i.e., growth and decline of an epidemic—but with the much more fundamental and difficult problem: "How does the first case appear?"

In several animal diseases the answer is clear. The disease agent may be carried latently as a subclinical infection in other animal species (bovine pseudorabies carried in pigs). In a second type the virus occurs in a host (e.g., dog) already infected with another parasite—a helminth. The virus apparently localizes in the helminth (fluke), for as the helminth progresses throughout the complicated phases of its life cycle, the virus accompanies it. The helminth (and virus) can finally appear in a food animal (salmon) and when the latter is fed to susceptible animals (dogs and other canidae), the helminth migrates through the tissues sowing virus in its wake. "Salmon poisoning" is then actually a virus disease, the virus having been seen and described since Shope's article appeared. In a third instance (swine influenza) the virus agent is present in the susceptible host, but inactive ("masked"); with the proper precipitating factors—bacterial infections, possibly meterological conditions—an epidemic starts, often from many foci, and thus explosively.

This work was all done with animals. Shope's thought is forceful and suggestive. It should have impact. If chauvinistic anthropophilism induces inertia, that will be unfortunate for the inert, their institutions, their science, and their patients.

A report by J. M. Pearce is concerned with certain viral infections in rabbits which were followed by cardiac lesions. The lesions (myocarditis, pericarditis, and endocarditis, sometimes with valvular vegetations) were greatly increased by the administration of various chemical substances or by reduced oxygen pressure. These methods all produced cardiac anoxemia. Virus localization was decided upon by the presence of characteristic inclusions. Pearce points out the implications for human disease processes and remarks very justly that failure to isolate viruses from infected human hearts may indicate chiefly that pathologists are not equipped for virus isolations.

Finally, two papers are detailed studies on the behavior of viruses in the animal body. F. Fenner has traced an acute infection, mouse pox, day by day, showing where multiplication takes place and how dissemination occurs. He believes that the human poxes may, like mouse pox, arrive in the skin after a period of multiplication in the internal viscera; e.g., liver and spleen, and subsequent hematic dissemination. J. G. Kidd discusses a great deal of material relating to the incitation of proliferative lesions by viruses, arriving at a rather cautious interpretation of "causation" in this connection. Results with the Shope papilloma virus, one of the best studied of the group, lead the author to consider it to be a carcinogen analogous to tar or methylcholanthrene and, like them, to play no essential part in the continuing malignancy of cancer cells.

The book is stimulating and provocative, and a review can only give a sampling of the contents. It is copiously illustrated with photomicrographs of unequal value, those of Dr. Howe being particularly fine. The tendency to abandon drawings for photographs nowhere shows to worse advantage than in articles detailing minutiae of cell structure and inclusions. It would seem to be wasted effort to apply photography to those structures for which photographic methods are not suitable. The editing of this volume was not overzealous; there is no index, and it is somewhat disconcerting to find the name of the editor incorrectly rendered.

DAVID WEINMAN

A Symposium on Steroid Hormones. Edgar S. Gordon, Editor Madison, The University of Wisconsin Press, 1950. 396 pp. $6.50.

This book is composed of the papers which were presented at a conference on steroid hormones at the University of Wisconsin in 1949. Principal attention was given to the chemistry, metabolism, and physiology of the sex and adrenal cortical steroids. The thirty-four contributors are experts in their fields, and the book constitutes a valuable series of summaries. The material on enzyme action in the metabolism of steroids and on avian endocrinology is particularly helpful; recent inclusive treatments of these subjects are difficult to find. The bibliographies appended to most of the chapters add greatly to the utility of the book. The only serious defect is the omission of an index. Format, printing, and binding are satisfactory.

T. R. FORBES

Principles of Ophthalmology. By Thomson Henderson. New York, Grune & Stratton, 1950. 230 pp. $4.50.

Although the title of this book would lead one to believe that it was one of basic general information in ophthalmology, the first few pages soon change this thought. It turns out to be a summary of some personal ideas of the author, ideas which he attempts to support through extended measurements of comparative anatomy of practically the entire known group of Mammalia. Through these comparative studies he attempts to disprove some of the theories which have become basic in the study of the function of the human eye. These studies take him into considerations of intraocular pressure, mechanism of accommodation, glaucoma, myopia, and papillaedema. From this it can be seen that this book is not intended for the beginner or the unacquainted in ophthalmology. To the reviewer's mind it is something to be read, thought over, and probably to a great extent discarded.

FRANCIS P. GUIDA

The Future in Medicine. New York Academy of Medicine, No. xiv of Lectures to the Laity. New York, Columbia University Press, 1950. x + 160 pp. $2.50.

Five lectures make up the contents of this book, viz. "Law and Medicine" by Robert B. Patterson, "The Endocrines: Servants or Masters" by Ephraim Shorr, "Science Under Dictatorship" by Leo Alexander, "Blood and Man" by William C. Boyd and "The Criminal Within Us" by George E. Gardner. Mr. Patterson's discourse is quite informal and chatty and adds little to the known problems between law and medicine. It is unfortunate that this was the first article, setting an initial rather dull and unscholarly tone to the book. Dr. Shorr's talk on the endocrine glands is a well-versed presentation of the development of the endocrines and their regulatory action in the maintenance of the internal environment. By far the most interesting and informative article to the medical reader (unless one has followed the original articles) is the dispassionate and well-documented essay by Dr. Alexander on "Science Under Dictatorship" in which he points out the conversion of medical scientists in Germany from ethical to highly unethical standards first by the practice of euthanasia and then

by the introduction of involuntary experimentation on human subjects. He concludes by pointing out that recent developments in the field of pain control no longer give euthanasia any moral basis whatsoever. In "Blood and Man" Boyd has tentatively classified man into six main racial groups according to blood types, and hence genetic frequencies. He believes that much more can be done along these lines with other genetically derived characteristics. The final lecture by Dr. Gardner on "The Criminal Within Us" exemplifies what Mr. Patterson suggested in the first lecture, namely, that the psychiatrist should look to his language. It might be stated parenthetically, however, that the jargon of psychiatry is exceeded in obscurity only by the jargon of law. Dr. Gardner has cleverly made several valid points in relation to the administration of justice which deserve considerable attention.

This book cannot be unhesitatingly recommended to the laity or to the medical profession, but Drs. Alexander and Gardner provide good material for off-moment reading.

WILLIAM D. BLAKE

CHARLES DARWIN'S AUTOBIOGRAPHY. Sir Francis Darwin, Ed. New York, Henry Schuman, Inc., 1950. 266 pp. $3.50.

The naturalist Charles Darwin is universally known; his chief work *Origin of Species* is a classic of science. Of the man, however, and his methods of working, probably unusually little is known even among biologists. Darwin was a retiring sort, troubled with almost lifelong chronic illness; his friendships were close, but not extensive, and he had almost no social life.

This volume, edited by his son Sir Francis, provides an intimate close-up of Darwin in his home and at work in his library, as well as a circumstantial account of the growth of the *Origin*, a labor consuming over twenty years. The materials are Sir Charles' short autobiography, written for his children and not for publication, Sir Francis' own notes on childhood memories of his father, and excerpts from many letters written during the growth of the *Origin*, chiefly to naturalist-geologists J. D. Hooker and Charles Lyell.

An introductory chapter by George Gaylord Simpson entitled "The Meaning of Darwin" purports to put Darwinism in perspective in scientific history and apparently to abstract the succeeding text. It contributes little to this book.

The chapters bearing Darwin's letters are particularly well edited. Whereas collections of letters often become boring through overinclusion of trivia, these have been carefully arranged and excerpted to lead the reader through the laborious evolution of the *Origin*. The circumstances of the joint reading with Wallace of the original paper before the Linnaean Society are fully brought out.

In an evening of relaxing reading one gains acquaintance with Darwin's friends and family, his forms of recreation, and his methods of work. Further, the reader acquires a deeper appreciation for the careful researches from which grew the *Origin*.

W. P. McN.

PROBLEMS OF MORPHOGENESIS IN CILIATES. THE KINETOSOMES IN DEVELOPMENT, REPRODUCTION AND EVOLUTION. By André Lwoff. New York, John Wiley & Sons, 1950. 103 pp. $2.50.

This book consists of two parts: first, detailed observations on the behavior of the blepharoplasts in ciliates; and, second, an attempt to relate these observations to current speculations on the regulatory mechanisms guiding cell organization.

Any writings of Dr. A. Lwoff attract attention because of his important contributions to microbiology. It is regrettable that the present monograph is so written as to be completely comprehensible only to those familiar with the technical vocabulary developed by E. Chatton and his collaborators, a vocabulary which has by no means won universal—nor even general French—acceptance.

DAVID WEINMAN

ESSENTIALS OF OPHTHALMOLOGY. By Roland I. Pritikin. Philadelphia, J. B. Lippincott Company, 1950. 544 pp.

The aim of this author is to give a fundamental and comprehensive coverage of the field of clinical ophthalmology for medical students and the physician in general practice. This is so well done that the book could also serve well as a review to the ophthalmologist preparing to teach or preparing for examinations.

There are omissions and faults, such as saying that fogging methods cannot be used in myopes. The tables on compensation in different states are good but too detailed in view of the flexibility of local laws.

The author's approach and his illustrations on optical principles, especially those illustrations in three dimensions, show a freshness not to be found in the standard and popular manuals of ophthalmology. Particularly good are the short and clear classification tables and summaries, such as those on foramina of the orbit and diseases of the retina. The section on malingering is excellent.

ROCKO M. FASANELLE

THE NEW YORK ACADEMY OF MEDICINE: ITS FIRST HUNDRED YEARS. By Philip Van Ingen. New York, Columbia University Press, 1949. 573 pp. $10.00.

This book is the biography of an institution written from its archives—from its diary, so to speak. The New York Academy of Medicine is one of the more important scientific societies, and this well-written, scholarly history portrays its extensive contributions to medical ethics, to the practice of medicine, and to the advance of medical knowledge for the period 1847-1947.

The book is written in a strictly chronological style which is adequate for its subject. A chronological arrangement always results in a discontinuity of sub-topics—there are about 275 page references under "Library" in the very complete index—but the arrangement is best for presenting the development of an institution as a whole.

Most important of all, Dr. Van Ingen has taken advantage of the opportunity to show the interrelationships between medicine and society. Much material for a case study of this topic is in his book.

FREDERICK G. KILGOUR

ELEMENTS OF HUMAN PHYSIOLOGY. By Miriam Scott Lucas. Philadelphia, Lea & Febiger, 2d edition, 1950. 357 pp. $4.75.

The second edition of this college text of human physiology shows very little change from the first edition. Despite the statement in the preface that the section on the nervous system is completely rewritten, there seems to have been no rewriting at all of this section, but merely a moderate rearrangement involving the bodily transposition of entire paragraphs. This is about the extent of any of the revisions of the text in the entire book. Of the only two real changes in this edition one is of a regressive nature, one is progressive, neither is very spectacular. The regressive change concerns the color plates of the first edition, all but two of which have shed their color, appearing in this edition in their primitive black-and-white. The other modification is the addition of references to additional reading, nonexistent in the first edition, at the ends of chapters. These bibliographies, however, are still in their infancy, being neither extensive nor particularly well selected. Such an obvious choice, for instance, as Fulton's *Physiology of the Nervous System* is omitted.

The coverage of topics is nominally large, but the actual treatment of most topics is quite brief, being either a broad, non-detailed account of the subject, or a fairly detailed exposition made compact through lack of logical development. The physical aspects of the book—binding, format, paper, printing, and illustrations—are all excellent. In fact, the 158 illustrations, mostly selected from the standard texts of anatomy, histology, and physiology, are the most satisfactory, albeit unoriginal, feature of this text.

R. G.

HARVEY CUSHING, SURGEON, AUTHOR, ARTIST. By Elizabeth H. Thomson. New York, Henry Schuman, Inc., 1950. xviii + 347 pp. $4.00.

Viewing with affectionate amusement the literary output of one of his young colleagues, Dr. Cushing once wrote to his friend, Arnold Klebs, that he thought there should be a sign above that colleague's door, "Obituaries written while you wait." In those later years of his life, he was also fond of saying that the thought of being the subject of such attention added one more terror to death. He heartily detested the current biographical trend of dissecting people, putting every thought and deed under the microscope and trying to read into them motives that were usually the product of the author's own imagination. In writing the biography of Sir William Osler, he had it clearly in mind that he must not make the man a saint (even though W. O. probably seemed a good deal like one to him) but at the same time that he must be "kind" without in the least distorting the truth.

All this is preamble to the statement that Dr. Cushing has been fortunate in his own biographers. Although in the two accounts of his life that have appeared since 1939 various incidents are outlined to which he very possibly would have given a slightly different interpretation, especially in Dr. Fulton's full-length biography, Dr. Cushing would have been the first to appreciate the spirit in which these accounts of his life had been written and to recognize the particular merits of Miss Thomson's portrayal—and would have been embarrassed though pleased to find himself in the distinguished company of Henry Schuman's Life of Science Library.

With a definitive "Life" of Dr. Cushing already published, Miss Thomson's task has been to portray her subject more briefly and with a non-medical audience particularly in mind. This has resulted, perhaps unconsciously, in greater emphasis on personal details than is true of the earlier biography; but the book having been written with ability and taste, there emerges a sympathetic account of the man's accomplishments against the background of his personality. Every reader—lay or medical, student or practitioner—will find its perusal most rewarding.

Coming from a line of pioneer physicians on his father's side, Harvey Cushing inherited resourcefulness and a "dogged as does it" determination that was to stand him (and his patients) in good stead throughout his life. From his mother he had a sweetness of character that was especially apparent in his contact with patients. In the letters sent to his family from camp and college, the outlines of the years ahead are already limned. His later life demonstrated the potency of "friendships formed at Yale," for not only did the pattern of the mould take form there, but all those persons and places remained important to him to the end of his days, and he returned to New Haven late in life with the comfortable feeling of going home. The years at Harvard Medical School saw the real hardening of the mould: the recognition that his profession must always be of primary importance, and the growing urgency to be "firstest" and "bestest."

One does not choose lightly a career in a branch of surgery with a frightening mortality. But having discovered his interests converging on the central nervous system, especially after his year abroad with Kocher, Kronecker, and Sherrington, Cushing set out after his return to Baltimore in 1901 to assemble and coordinate all known facts about the brain and spinal cord and by careful and complete study of every case to amass a body of material that would eventually reveal some of the body's hidden secrets—and drastically reduce the early mortality percentages. To this end he rigorously imposed on his residents the necessity of detailed histories, even the recording of all negative findings; in the years to come these proved their value many times over. He drove himself hard throughout his professional life, and none of his assistants found his service an easy one. A stern taskmaster, he constantly maintained a fierce discipline —*un discipline de fer*, to use the phrase of the late Clovis Vincent—and viewed with distaste the casual intrusion of personal relationships on the professional scene. He had a great feeling for the fitness of things, and without wasting words saw to it that those around him were aware of that feeling. He could, however, be a most charming and diverting companion in his periods of relaxation, and everyone who had the good fortune to know him cherishes memories of those times when his gay humor and wit were allowed the upper hand.

In Miss Thomson's book the impact of Dr. Cushing's forceful character on the individuals and institutions with which he came in contact is woven into the story of his life, as is the part that Mrs. Cushing played throughout the years—not an easy one, but carried out with spirit and in the manner of the great lady that she was. The two busy, exciting decades in Boston that witnessed phenomenal strides in the field of neurological surgery, the growth of a great clinic with an ever-increasing number of students from this country and abroad, the years of retirement at Yale and the careful strengthening of an already magnificent library—all are described with pleasant simplicity. From the wealth of Cushing memora-

bilia in the Historical Library Miss Thomson has made happy selections. Her felicitous choice of chapter titles and her crisp manner of ending each chapter provide added pleasure for her readers.

It is now eleven years since Dr. Cushing's death. The two full-length biographies that have appeared in this interval have given us the story of his life and personality, but neither author has made a direct attempt to place him in his surgical time and evaluate his contribution to the advancement of neurosurgery. It is to be hoped that the next study of his life (if such there may be) will be undertaken by someone who can do this. One thinks inevitably of Sir Geoffrey Jefferson in this connection.

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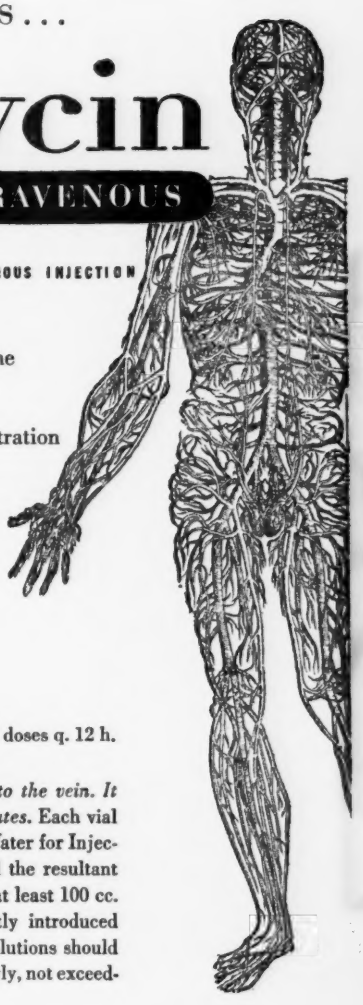

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